RELATIVE INFREQUENCY OF SOYBEAN VARIETIES HAVING ONLY ONE FACTOR FOR YELLOW COTYLEDON[1]

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In the soybean there are two cotyledon colors, yellow and green. Both types are green in immature soybeans, but as the beans ripen, the green pigment fades out in yellow-cotyledon varieties, and persists in green-cotyledon varieties. In inheritance, yellow differs from green in possessing a factor or factors that cause the green pigment to fade out as the beans mature.

In crosses between yellow and green cotyledon types, yellow proves to be dominant, and in F_2, a ratio of 3 yellow to 1 green, or 15 yellow to 1 green, is obtained according as one or two (duplicate) factors, D_1 and D_2, are involved.

In a former paper (WOODWORTH 1921) it was stated that while all the data available indicated that yellow color of the cotyledons was dominant to green, yellow cotyledon varieties differed among themselves with regard to the factors D_1 and D_2 that cause the green pigment to disappear at maturity. "Some, perhaps most varieties, as, for example, Auburn, possess only one factor of this nature, while others typified by Variety 8, possess two such factors." It now appears that the reverse of this statement is true, that is, that most yellow-cotyledon varieties possess two factors instead of only one. This is borne out by a study of the results secured to date from crosses showing segregation in cotyledon color (table 1). Of the 27 varieties listed in table 1, 23, or eighty-five percent, possess two factors for yellow cotyledon.

In contrast with this condition in soybeans, SHULL (1918) finds that types of shepherd's purse having duplication of a leaf-lobe factor are less frequent than those having only one such factor. From this fact he argues that "the duplication of this factor has taken place at a relatively recent date." Taking the same line of reasoning we may say that duplication of cotyledon factors in the soybean probably occurred at a remote date. The wild soybean, which is supposed to be the ancestor of our common

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types, has yellow cotyledons. The constitution of the wild soybean with respect to D_1 and D_2 has not been determined experimentally. However, the large number of common varieties descended from it and known to have both factors, is an indication that it also contains both. If soybeans with green cotyledons have arisen from the yellow by mutation, we should expect that single factor changes would occur more frequently

TABLE 1

Summarizing results of experiments on soybean crosses involving segregation in cotyledon color.

YELLOW COTYLEDON VARIETY	F_2 RATIO YELLOW TO GREEN COTYLEDON	YELLOW COTYLEDON VARIETY	F_2 RATIO YELLOW TO GREEN COTYLEDON
Mandarin[3]	14.3:1	Progeny 2214	11.7:1
Aksarben[3]	15.0:1	Progeny 2218	15.2:1
Ito San[3]	17.0:1	Progeny 2225	18.6:1
Jap. Variety[3] (J_1)	16.2:1	Progeny 2235	16.6:1
Manchu[3]	9.3:1	Progeny 2247	17.8:1
Green (C_1)[3]	18.8:1	Progeny 2261	17.3:1
Variety 8	7.9:1	Progeny 2265	14.9:1
Midwest	16.5:1	Progeny 2266	16.8:1
S. P. I. 65345	17.6:1	Progeny 2269	14.1:1
Ilsoy	10.5:1		
Wea	15.6:1	Auburn	3.2:1
Progeny 2199	11.3:1	Mikado	4.1:1
Progeny 2208	24.3:1	Wilson	3.8:1
Progeny 2213	18.8:1	Progeny 2262	3.1:1

[3] Data from OWEN (1927).

than changes involving more than one locus at a time. The occurrence of single-factor changes would result in the production of one-factor varieties as an intermediate stage from two-factor types to green. The scarcity of one-factor types, however, points to the conclusion that green-cotyledon varieties, if produced by mutation from the yellow, are the result, in most cases, of simultaneous changes in duplicate factors.

The occurrence of duplicate factors for a character can be accounted for by a doubling of chromosome number (tetraploidy) or by a doubling of only the particular pair of chromosomes carrying the original factor. In either case we should expect that other factors carried on the chromosomes involved would show duplication. In the problem under discussion, the factor *G* for green seed coat color, is linked with one of the factors for cotyledon color, either d_1 or d_2, with about 12.5 percent crossing over. If a doubling in chromosome number were responsible for duplication of cotyledon factors, we should expect to find duplicate factors for green seed coat color, one linked with d_1, the other with d_2. So far as known, there is no duplication of factor *G*.

Another possibility suggested by SHULL (1918) is the mating of non-homologous chromosomes. This would result in duplication, but also in the production of a single factor type through segregation and recombination. It would seem, therefore, that on this hypothesis the number of one-factor types would at least equal the number of two-factor types.

It appears that in nature there is very little or no natural crossing between yellow and green cotyledon varieties; for such crosses would lead to the production of one-factor yellow varieties as well as two-factor yellow varieties. It is a simple matter to produce one-factor varieties by isolating the segregates from hybrids heterozygous for two factors. Such hybrids produce both yellow and green cotyledon beans in a 15 to 1 ratio, and when the yellows are planted, we expect that 7 will breed true for yellow, 4 will segregate in a 3 to 1 ratio, and 4 in a 15 to 1 ratio. To establish a one-factor variety, we have only to test the yellows of a 3 to 1 ratio plant. Those that breed true for yellow are homozygous for one factor only. In every cross involving two factors, there should be two such types, one carrying D_1, the other carrying D_2. It is clear that if many crosses occurred between two-factor yellows and greens, many one-factor varieties would be produced. The infrequency of one-factor yellows in nature is therefore an argument against the occurrence of such natural crosses.

Simultaneous mutations involving duplicate genes at the same time are believed to be too rare to account for the number of green cotyledon varieties found in nature. Furthermore, some of these varieties show maternal inheritance of cotyledon color when crossed with yellows (TERAO 1918; PIPER and MORSE 1923; and OWEN 1927) and must, therefore, have been produced in some other way than by mutation. Also, the evidence is against the occurrence of many natural crosses between yellow and green varieties. There appears to be at present, therefore, no satisfactory explanation for the small number of one-factor yellow varieties as compared with the number of two-factor yellow varieties of soybeans.

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CHROMOSOME NUMBERS IN FUNCTIONING GERM CELLS OF SPECIES-HYBRIDS IN WHEAT[1]

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INTRODUCTION

The second and later generations of hybrids between tetraploid and hexaploid species of wheat have chromosome conditions which are very different from those which are to be expected on the basis of the numbers in F_1 gametes. The parental numbers appear far too frequently in F_2, and the intermediate numbers disappear in later generations. The reasons for these differences from the expected cannot be satisfactorily determined by the study of ordinary hybrids alone. But additional information, particularly in regard to the chromosome numbers of those gametes which are capable of functioning, can be obtained from the study of backcrosses.

KIHARA (1919, 1924) and SAX (1922a) have shown that in the F_1 the 14 chromosomes from the tetraploid parent (emmer series) mate with 14 from the hexaploid parent (*vulgare* series), leaving 7 from the latter unmated. These 7 univalents fail to divide at the homotypic division and move at random to either pole. The chromosome numbers of the gametes therefore vary between 14 and 21, and the frequencies of the various numbers should be 1, 7, 21, 35, 35, 21, 7, 1. Consequently the great majority of plants in F_2 and in later generations should have intermediate numbers.

But several authors have shown that segregates with 28 and 42 chromosomes (somatic) are numerous, that many others have 29, 30, 40 or 41,

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and that the proportion of plants with approximately 35 is very small in comparison with expectations (KIHARA 1921, 1924; SAX 1923; THOMPSON 1925; THOMPSON and HOLLINGSHEAD 1927). Furthermore those intermediate numbers which were present in F_2 were shown to disappear in later generations. In addition KIHARA (1924) found that the chromosome formula of plants with more than 14 bivalents must be written $(14+x)$ bivalents $+(7-x)$ univalents. If any plant has more than 14 bivalents it has also enough univalents to make the total number 21. Such combinations as 15 bivalents plus 0 to 5 univalents, 16 plus 0 to 4, etc., do not appear, or only very rarely, although they should be expected fairly frequently, particularly in certain F_3 progenies.

Different suggestions were made by the authors mentioned to account for these results. SAX thought that they were due to the failure of the intermediate classes of gametes to function owing to chromosome unbalance. KIHARA attributed them rather to the failure of certain classes of zygotes to develop. There are other possible factors such as the loss of univalent chromosomes through lagging, chromosome conditions in the endosperm, some peculiar method of chromosome distribution, or some favorable relationship between chromosome numbers in male and female cells.

In order to decide the exact cause, it is necessary to know the chromosome constitution of the gametes which are capable of functioning. By means of counts in F_2, it is possible to determine the total number in the 2 gametes which unite to produce an F_2 plant but not the number in individual gametes. But by using backcross material this information can be obtained. When the chromosome number of the backcross has been determined, and that of the pure parent is known, that of the F_1 gamete which functioned can easily be inferred.

KIHARA (1925) has reported some results of backcrossing *T. Spelta* × *T. polonicum* F_1 male with the pure parents. Of the backcross with *polonicum* 4 plants were examined and all were shown to have resulted from the functioning of F_1 male gametes with 14 chromosomes. Of the backcross with *Spelta*, 10 plants were examined and 8 were shown to have resulted from the functioning of F_1 male gametes with 19 to 21 chromosomes. Since *polonicum* has 14 and *Spelta* 21, KIHARA concluded that the pollen grains which function are those which have a chromosome number similar to that of the female. It is possible, for example, that a 14-chromosome pollen tube may not develop as well as a 21-chromosome tube in a stigma of a plant with 21 chromosomes.

WATKINS (1927) has reported counts on backcrosses with *turgidum* ×

vulgare which lead him to conclude that all F_1 female gametes are capable of functioning, but that there is much elimination of male gametes.

METHODS

All chromosome counts were made on pollen mother-cells of stamens fixed in a mixture of absolute alcohol and acetic acid (2:1) and examined in aceto-carmine. Such preparations are much better than paraffin sections for the study of univalents, though usually not for total counts if the numbers are large. In this case the determination of the number of univalents is all that is necessary since if the backcross is with the 14-chromosome parent the number of univalents is the same as in the F_1 gamete which functioned (all 14 from the pure parent mating with 14 from the F_1 gamete); and if the backcross is with the 21-chromosome parent, the number of univalents is 7 minus the number in the F_1 gamete (each univalent from the latter finding a mate among the extra 7 chromosomes from the *vulgare* parent). Nevertheless, whenever it was possible—and this was the case in nearly every plant—the total number of bivalents and univalents was also determined for confirmation.

The number of univalents can be determined most easily and accurately at the heterotypic anaphase since they lag behind the bivalents, and since in aceto-carmine preparations of fixed material, side views are usually obtained. The number visible in the different pollen mother-cells of the same plant varies somewhat owing to premature splitting, failure to move into the equatorial position, or entanglement with the bivalents. But when any considerable number of cells is available for observation the true number appears in a large proportion of cases, as can easily be confirmed from total counts. At heterotypic metaphase, although most of the univalents are off the plate and can be recognized, some of them may be at the edge so that accurate determination of them is more difficult than at anaphase, though usually not impossible. In a few cases a small proportion of the cells showed 1 or 2 univalents more than was definitely determined to be the true number. This was apparently due to the occasional failure of 1 of the primary 14 pairs to mate. Many of the counts were made on material raised in the greenhouse during the winter of 1926–27, and the rest on material raised in the field in 1927.

Four kinds of F_1 plants were used: *dicoccum-vulgare* 1, *durum-vulgare* 1, *durum-vulgare* 2, and *dicoccoides-vulgare* 1. *Vulgare* 1 is the variety Marquis; *vulgare* 2 is of Chinese origin; the *dicoccum* variety is a white spring emmer; the *durum* is Iumillo; the *dicoccoides* is wild emmer. The *vulgares* have 21 chromosomes and each of the others 14.

EXPERIMENTAL DATA

Table 1 gives the cytological findings for a number of kinds of back crosses. It shows the frequencies of the F_1 gametes, with the various numbers of univalents, which functioned to produce the backcrosses. The total number of chromosomes in these gametes is obtained by adding 14 (the bivalents) to the numbers 0 to 7 in the first line of the table. Both male and female gametes of each of the 4 kinds of F_1 were investigated by using F_1 as both pollen and seed parent in the backcrossing. Also in the case of the *durum-vulgare* F_1, backcrosses were made with both *durum* and *vulgare* parents. The data on the F_1 gametes which functioned in all these kinds of backcrosses are given in the table.

In the backcrosses with *vulgare*, the number of univalents actually seen in each case was 7 minus the number shown, since each univalent in the F_1 gamete would mate with one of the 7 extra chromosomes from the *vulgare* parent and the rest of the 7 would be left unmated.

A study of table 1 brings out certain conclusions which may now be discussed in order.

TABLE 1

Frequencies of functioning F_1 gametes with various numbers of univalent chromosomes.

BACKCROSS	UNIVALENTS IN F_1 GAMETES								TOTALS
	0	1	2	3	4	5	6	7	
1. *Dicoccum* ♀ × (*vulgare* 1-*dicoccum*) F_1 ♂	18	8	2	3	2	0	2	2	37
2. *Dicoccum* ♂ × (*vulgare* 1-*dicoccum*) F_1 ♀	10	6	5	6	2	0	0	0	29
3. *Durum* ♀ × (*vulgare* 1-*durum*) F_1 ♂	12	7	4	2	0	0	1	3	29
4. *Durum* ♂ × (*vulgare* 1-*durum*) F_1 ♀	14	3	4	2	4	2	1	2	32
5. *Durum* ♀ × (*vulgare* 2-*durum*) F_1 ♂	3	2	0	1	2	2	0	5	15
6. *Durum* ♂ × (*vulgare* 2-*durum*) F_1 ♀	1	1	3	6	4	2	2	1	20
7. *Vulgare* 1 ♀ × (*vulgare* 1-*durum*) F_1 ♂	8	4	2	1	1	1	2	5	24
8. *Vulgare* 1 ♂ × (*vulgare* 1-*durum*) F_1 ♀	5	2	3	2	2	3	0	0	17
9. *Vulgare* 1 ♀ × (*vulgare* 1-*dicoccoides*) F_1 ♂	2	1	2	1	0	1	1	1	9
10. *Vulgare* 1 ♂ × (*vulgare* 1-*dicoccoides*) F_1 ♀	2	1	1	2	1	1	0	0	8
11. *Vulgare* 2 ♂ × (*vulgare* 2-*durum*) F_1 ♀	0	0	2	2	3	1	0	1	9
Expected proportions	*1*	*7*	*21*	*35*	*35*	*21*	*7*	*1*	

THE FAILURE TO FUNCTION OF MANY GAMETES WITH INTERMEDIATE CHROMOSOME NUMBERS

The last line of the table shows the proportions of the various classes of gametes which are to be expected if all are capable of functioning. The actual proportions are very different in every case. The most conspicuous difference is the small number of gametes with intermediate numbers (3, 4, and 5). For example, the ratio of gametes with 0 to those with 3 is expected to be 1:35, whereas it is actually 18:3, 10:6, 12:2, etc. A result of this kind would be anticipated from the work of several investigators on F_2. SAX (1923) believes that nearly all F_1 gametes with intermediate numbers are sterile. KIHARA (1924), on the other hand, thinks they are all capable of functioning but that their failure to produce offspring is due to zygotic mortality. These results strongly favor the theory of gametic sterility. Nevertheless a high enough proportion of them is capable of functioning to produce many F_2 plants. Presumably their usual failure to function is due to the bad effects of an unbalanced chromosome number. Whether they actually die or are merely unsuccessful in competition with the others is not determined. The percentage of visibly abortive pollen in F_1 is not high enough to account for these figures.

In a later publication (1925) KIHARA reported data which indicated that the functioning of the pollen grain may depend on the relation of its chromosome number to that of the female plant. When *Spelta*×*polonicum* F_1 male was backcrossed with *polonicum* female, all the 4 F_1 pollen grains which functioned to produce the plants examined, had 14 chromosomes; but when the backcross was with *Spelta* 8 of the 10 grains which functioned had from 19 to 21 chromosomes. KIHARA concluded that the pollen grains which function are those which have a chromosome number similar to that of the female. Failure to function may result from poor growth of the pollen tube in a stigma which is unsuitable because of its chromosomes.

Our results do not support this conclusion. In the backcross with *durum* female (line 3 of table 1) 19 out of 29 grains had 14 or 15 chromosomes and 4 had 20 or 21; in the backcross with *vulgare* female (line 7) 12 out of 24 grains had 14 or 15 and 7 had 20 or 21. Grains with the higher numbers were, therefore, only slightly more successful on *vulgare* than on *durum* stigmas. Half the grains which were successful in the *vulgare* backcross had only 14 or 15 chromosomes.

PREPONDERANCE OF 14- OVER 21-CHROMOSOME GAMETES

Chromosome unbalance will not account for the great preponderance, in nearly every case, of gametes with no univalents, or only 1, over those

with 6 or 7. It will be observed from the table that the ratio of gametes with 0 univalents to those with 7 in the different cases is 18:2, 10:0, 12:3, etc., whereas it should be 1:1. This is doubtless due, in part at least, to the loss of chromosomes through lagging at the reduction divisions. These laggards frequently fail to become incorporated in the new nucleus and degenerate in the cytoplasm. (For the frequency of this phenomenon see THOMPSON and HOLLINGSHEAD 1927). Intermediate numbers would thus tend to become low, and high numbers to become intermediate (a condition which rarely functions). Whether this is the correct explanation or not, there are certainly far more functional gametes with 14 than with 21 chromosomes. This results in the great preponderance of emmer types in F_2 noted by THOMPSON and HOLLINGSHEAD in *dicoccum-vulgare* and to a less extent by THOMPSON (1925) in *durum-vulgare* hybrids.

One exception to this rule should be noted. When *vulgare* 2 is used in making *durum-vulgare* crosses, the F_1 gametes with 7 univalents are as numerous as those with 0 or more numerous.

DIFFERENCES BETWEEN MALE AND FEMALE GAMETES

Another important point evident in the table is that male gametes with intermediate numbers are eliminated in greater proportions than are female gametes with the same numbers. In nearly every case there is a smaller proportion with intermediate numbers when the F_1 is the male parent than when it is the female parent. This may be due to the greater competition among the numerous male gametes, only the stronger ones usually functioning. In no case, however, are all the males with intermediate numbers eliminated. And in no case are all the female gametes functional. In every case in which the F_1 was the female parent, the proportions of gametes with intermediate numbers are less than the theoretical. The only case in which the expected numbers are approached is where *vulgare* 2 was one parent.

This result is quite different from that obtained by WATKINS (1927) with *vulgare*×*turgidum* hybrids. He is of the opinion that all female F_1 gametes in that cross are capable of functioning. The correctness of our conclusion, for our material at least, is confirmed by comparing the percentage of seed set in backcrossing where the F_1 was the female parent, with that set in straight crossing. The figures in this connection are given in table 2. In every case the percentage of seed set is considerably less in the backcross, showing that a higher proportion of the eggs of F_1 than of the pure parent fails to function. These crosses were all made in the same year by the same person.

TABLE 2

Percentages of pollinated flowers setting seed.

Vulgare-durum F_1 ♀ × *durum* ♂	34.46
Vulgare-durum F_1 ♀ × *vulgare* ♂	33.96
Vulgare-dicoccum F_1 ♀ × *dicoccum* ♂	33.40
Vulgare-dicoccum F_1 ♀ × *vulgare* ♂	34.44
Vulgare ♀ × *durum* ♂	72.87
Vulgare ♀ × *dicoccoides* ♂	59.21
Dicoccum ♀ × *vulgare* ♂	84.33

DIFFERENCES AMONG THE KINDS OF CROSSES

It is also evident from table 1 that somewhat different results are obtained with different kinds of F_1. For example there is a greater elimination of intermediate numbers in *vulgare-dicoccum* than in other F_1's. When *vulgare* 2 is used in crossing with *durum* the results are very different from those obtained when *vulgare* 1 is used. In the former case there is much less elimination of intermediate numbers. Its female gametes (backcrosses number 6 and 11) approach the expected much more closely than any of the others and both male and female gametes have 6 or 7 univalents much more frequently than do those of other crosses. Not only the kind of F_1 but also the kind of backcross is important. The elimination of both intermediate and high numbers of univalents is less when the backcross is made to *vulgare* than when it is made to *durum*. All these differences emphasize the danger in drawing sweeping conclusions from work with a limited variety of material. In the difficult problems of practical breeding with wheat, what is found to be impossible to accomplish with one or several crosses, may prove quite feasible with others.

CHROMOSOME NUMBERS IN RELATION TO ENDOSPERM DEVELOPMENT AND SEED MORTALITY

Certain aspects of these results have been determined or influenced by differences in the germinating capacity of different classes of seeds. This is illustrated in table 3 which shows the percentages of germination of grains from certain reciprocal backcrosses as well as from straight crosses. The grains recorded are those produced directly on the plants which were artificially pollinated, not on their offspring. It is clear from these data that in backcrosses with the 14-chromosome parent the germination is better, in some cases much better, when the F_1 is the female, than when it is the male parent. Even in straight crosses there is a

marked difference in germination depending on the direction of the cross. If the 21-chromosome parent is the female the germination is much higher than if it is male.

TABLE 3

Comparative germination in reciprocal crosses and backcrosses.

	PLANTED	GERMINATED	PERCENT GERMINATED
Dicoccum ♀ × F_1 ♂	72	48	66.6
Dicoccum ♂ × F_1 ♀	67	52	77.6
Durum ♀ × F_1 ♂	40	21	47.5
Durum ♀ × F_1 ♀	31	28	90.4
Vulgare ♀ × F_1 (*vulgare-durum*) ♂	29	26	89.6
Vulgare ♂ × F_1 (*vulgare-durum*) ♀	38	23	60.5
Vulgare ♀ × F_1 (*vulgare-dicoccoides*) ♂	17	17	100.0
Vulgare ♂ × F_1 (*vulgare-dicoccoides*) ♀	22	12	54.5
Durum ♀ × *vulgare* ♂	93	51	55.9
Durum ♂ × *vulgare* ♀	19	16	84.2
Dicoccum ♂ × *vulgare* ♀	10	8	80.0
Durum ♀ × *Spelta* ♂	24	8	33.3

Note: These data are for directly crossed seeds, not for seeds borne on plants resulting from crossing. The seeds recorded in the first 4 sections of the table would produce backcross plants; those in the last 2 sections would produce F_1.

These differences in germination depend directly on the different degrees of endosperm development which are evident from the varying amounts of shrivelling of the seeds. And the endosperm development depends on chromosome conditions, as has been pointed out by WATKINS (1927). The chromosome number in the endosperm of seeds resulting from crossing a 21- with a 14-chromosome wheat will depend on the direction in which the cross is made. If the 21-chromosome plant is the female, the endosperm will have $56(21\times2+14)$ chromosomes since it is formed from the fusion of 2 female nuclei (each with 21) and a male nucleus (with 14). But if the 21-chromosome plant is the male, the endosperm will have $49(14\times2+21)$. In the one case the extra 7 *vulgare* chromosomes are diploid and in the other haploid. From the data on straight crosses given in table 3, it is clear that germination is much better when the 7 are diploid. Similar considerations must be kept in mind in discussing F_1 and plants which may have between 14 and 21 chromosomes.

TABLE 4
Chromosome numbers and endosperm development.

		LARGE PLUMP	SMALL PLUMP	LARGE WRINKLED	SMALL WRINKLED	SHRIVELLED	TOTALS
Vulgare 1 ♀ × *vulgare* 1-*durum* F_1 ♂	Frequency	32	48		10	7	97
	Planted	10	17		2		29
	Died	2	1		0		3
	Univalents in F_1 gametes	7, 7, 7, 7, 7, 6, 6, 5	4, 3, 2, 2, 1, 1, 1, 0, 0, 0, 0, 0, 0		1, 0		
Vulgare 1 ♂ × *vulgare* 1-*durum* F_1 ♀	Frequency	11	2	44	12	20	89
	Planted	8	2	23	5		38
	Died	0	1	10	4		15
	Univalents in F_1 gametes	2, 3, 4, 4, 5, 5		0, 0, 0, 0, 0, 1, 2, 2, 2, 3, 4			
Vulgare 2 ♂ × *vulgare* 2-*durum* F_1 ♀	Frequency	8	5	26	16	21	76
	Planted	7	5	10	3		23
	Died	0	0	6	3		9
	Univalents	3, 4, 5, 5, 7	4, 4	2, 2			
Vulgare ♀ × *vulgare-dicoccoides* ♂	Frequency	10	14				24
	Planted	8	9				17
	Died	0	0				0
	Univalents	3, 4, 5, 6, 7	0, 0, 1, 2				
Vulgare ♂ × *vulgare-dicoccoides* ♀	Frequency	1		15		15	31
	Planted	1		14		7	22
	Died	0		4		4	8
	Univalents			0, 0, 3, 3, 4, 5		1, 2	
Durum ♂ × *vulgare-durum* ♀	Frequency	24	15				39
	Planted	8	3				11
	Died	0	0				0
	Univalents	0, 0, 1, 2, 6	4, 4				

Information regarding the interrelationships between chromosome numbers, the direction of crossing, endosperm development, and germination is given in table 4. If one compares the first two sections of the table, it is seen that in backcrossing with the 21-chromosome parent, the proportion of wrinkled and shrivelled seeds obtained is low when the F_1 is the male parent but very high when F_1 is the female. In the former case (*vulgare* ♀ × F_1♂) the endosperm has the extra 7 diploid plus whatever number of univalents the male contributes, which is usually 0 *or* 7. And such endosperm is almost always well developed. Therefore the endosperm develops well if the 7 are exactly diploid or triploid or nearly so. From the chromosome numbers determined (bottom of first section of table 4) it is also evident that the triploid condition gives better endosperm than the diploid, since when the male contributes 6 or 7 univalents the seeds are large, whereas when it contributes 0, 1 or 2, they are small (though not shrivelled).

In the reciprocal of this cross (F_1 ♀ × *vulgare* ♂; second section of the table) the endosperm contains in the diploid condition whatever chromosomes are contributed by the F_1 (usually less than 7) plus the complete set of 7 contributed by the *vulgare* male. Consequently the endosperm is usually haploid for all 7 (when F_1 contributes 0 univalents) and frequently triploid with respect to some chromosomes and haploid with respect to the rest. Under these conditions endosperm development and germination is usually poor. Moreover the actual chromosome counts (bottom of second and third sections) show that the small proportion of round seeds do have the triploid condition of most of the chromosomes while the wrinkled ones have the haploid.

A similar situation is seen in the backcross of *vulgare-dicoccoides* with *vulgare*.

When the F_1 is backcrossed with the *durum* the seeds are plump. In this case the extra 7 *vulgare* chromosomes are rarely represented at all in the endosperm, since the F_1 usually contributes none, and of course the *durum* has none. Therefore the endosperm is like that of pure *durum*.

All these cases are in line with a general rule which may be stated as follows: The endosperm is well developed when it is (a) entirely without the extra 7 *vulgare* chromosomes, (b) when it has either 2, or, (c) better still 3 complete sets. It is likely to be badly developed when it is (a) haploid for all 7 or (b) diploid or triploid for some only of the 7 or (c) diploid of triploid for some and haploid for others. In general the endosperm appears to be affected in the same way as the gametes by unbalanced chromosome numbers; the unbalance may be even greater in the endosperm.

A number of important applications of these conclusions may be pointed out. In ordinary crossing one obtains many more F_1 plants for the same amount of labor if *vulgare* is the female than if it is the male parent, since in the latter case the endosperm is haploid with respect to the seven. This is important in practical breeding where a large F_2 may be required. Again it is well known that certain wide crosses, for example wheat×rye, wheat×Aegilops, *vulgare*×*monococcum*, etc., can be made in only one direction. The species with the larger chromosome number must be used as the female, in order to give an endosperm which is diploid for the extra chromosomes.

The conclusions reached above make it probable also that the F_2 and later generations usually raised are not fair samples. There is a tendency to select the better looking seeds for planting and to leave the shrivelled ones or to take no special care with them. But the embryos of the better seeds are likely to have either 0 or all 7 of the extra chromosomes while the shrievelled ones are likely to have the intermediate numbers. It is therefore probable that the lack of intermediate chromosome numbers is due in part to the conscious or unconscious selection of the better seeds for planting. Of course a proportion of the poor seeds would fail to germinate in any case, particularly if conditions were not favorable. Nevertheless the frequency of plants with intermediate chromosome numbers would be increased by giving special attentation to the poorer seeds. And it is probable that such plants are the ones most likely to possess the combinations of characters which the practical breeder desires.

SAX (1922b) has reported some results which are in agreement with this conclusion. In a cross between Kubanka and Bluestem he found that a majority of F_2 seeds (borne on F_1 plants) which failed to germinate were in the lower weight classes. He also found a correlation of 0.24 between wrinkling of F_2 seeds (borne on F_1) and sterility of F_2 plants which produced heads. He could find no correlation, however, between wrinkling and size of F_2 plants.

It is probable that the frequency of intermediate numbers in table 1 would have been increased by selection of wrinkled seeds and special care in their germination. These facts about the effect of chromosome numbers in endosperm development and germination were not realized when the seeds of some of the backcrosses were planted. In some cases, however, all the seeds available were planted, but no special care was taken with the shrivelled ones.

BEARING ON RESULTS PREVIOUSLY REPORTED FOR F_2 AND LATER GENERATIONS

The chromosome numbers in F_2 and later generations as reported by SAX (1923), KIHARA (1924), THOMPSON (1925), THOMPSON and HOLLINGSHEAD (1927), are in general such as would be expected if the F_1 gametes which are capable of functioning have the chromosome numbers reported in this paper. One discrepancy is a frequency of plants with intermediate numbers lower even than our figures would indicate. This may be explained in part by the selection of the better seeds for planting. It may also be due in part to the greater effect of chromosome unbalance in the endosperm in the ordinary hybrids than in the backcrosses. In the latter one parent contributes 1 or 2 complete sets of the 7 or none at all, whereas in the former the F_1 makes all 3 contributions of intermediate numbers to the endosperm. The effect of chromosome unbalance is therefore likely to be greater in the endosperm of ordinary hybrids than in that of backcrosses.

Another marked discrepancy is the absence from F_2 of any plants with more than 14 bivalents and less than 21 as the sum of bivalents and univalents. Such combinations as 15 bivalents plus 0–5 univalents, 16 plus 0–4, etc., do not appear or only very rarely. They should be found if the gametes have the numbers shown in table 1. They should not be very frequent in F_2 but they should occur, and they should be fairly frequent in certain F_3 families. This study shows that their absence is not due entirely to gametic elimination. It must be due also to zygotic mortality. Apparently zygotes having more than 14 bivalents without having enough univalents to make the total 21 are not viable. They may die as a result of endosperm abortion. They could result only if both gametes had an intermediate number of univalents. Therefore the endosperm could not be triploid or even diploid for all 7 but would be triploid for some of the 7. For example if the pollen grain had chromosomes a and b of the extra 7, and the egg had a, b and c, then the endosperm would have 3 a's, 3 b's and 2 c's, and none of the others. It has been shown from the data on backcrosses that such an endosperm would be severely shrunken and the seed probably fail to germinate or die early. Hence the non-appearance of such combinations may be due to endosperm abortion. Of course the young zygotes may die from other causes.

SUMMARY

1. An effort has been made, by the study of backcrosses, to determine the causes of the cytological conditions which have been reported for

hybrids between 14– and 21–chromosome wheats. The study of backcrosses makes possible the exact determination of the chromosome numbers of F_1 gametes which are capable of functioning. This information is not obtainable from the study of ordinary crosses. Four kinds of F_1 were backcrossed reciprocally so that both male and female gametes could be investigated and both 14- and 21-chromosome parents were employed in making the backcrosses.

2. In nearly every case the gametes with numbers of extra *vulgare* chromosomes intermediate between 0 and 7 were in much smaller proportions than were to be expected, if all gametes are capable of functioning.

3. In most cases the gametes with no extra univalent chromosomes (14 in all) were much more numerous than those with 7 (21 in all).

4. There are marked differences between the male and female gametes of F_1, the latter showing less elimination of intermediate numbers than the former, though by no means all eggs are capable of functioning and a proportion of functioning pollen grains do have intermediate numbers.

5. There are marked differences between the different kinds of hybrids with respect to their functional gametes, and slight differences depending on whether a hybrid was backcrossed with the *vulgare* or with the emmer-type parent. It is dangerous to draw sweeping conclusions from a limited variety of material.

6. The endosperm is likely to be poorly developed (and the seeds shrivelled) unless its extra 7 *vulgare* chromosomes are completely absent, or completely diploid, or completely triploid. The farther it departs from these conditions the greater is the shrivelling. Reciprocal crosses may, therefore, give very different results and wide crosses may be possible in only one direction. Unless special care is taken of the shrivelled seeds in species-crosses a fair sample is not obtained in F_2 since the shrivelled ones contain chromosome combinations not likely to be found in plump ones.

7. If gametes with the frequencies found mate at random, the results should be in general like those which have been reported for F_2. Discrepancies are seen in the lower frequencies of intermediate numbers and especially in the absence of combinations of more than 14 bivalents with less than enough univalents to make 21 altogether. The absence of these may be due to zygotic weakness but may also be attributed to endosperm abortion.

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INHERITANCE OF EARLOBE COLOR IN POULTRY[1]

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INTRODUCTION

The earlobe of the chicken is a structure on the skin of the face just below the ear, the outline of which is marked by a slight thickening of the tissues. It is bare of feathers and may be the same color as the rest of the face for which the degree of redness is somewhat dependent upon the health of the bird. As is true of most of the head furnishings, this structure is larger in males than females.

For some breeds of poultry, more commonly those classified as Mediterraneans, the color of the earlobe differs from that of the rest of the skin of the face. In such cases it is of a pearl white color and although the histology of the tissue has not been studied the color appears to be due to a deposition of white material just beneath the epidermal layer. In the White-Faced Black Spanish breed the white color is extended to cover the entire face. In the breeds of the Mediterranean group the earlobe is proportionately larger than in most other breeds.

The poultry fancier has decreed that the earlobe must be all white or all red, making the color a breed characteristic. Since even slight variations from standard color constitute a show ring disqualification, uniformity of color has been rigidly selected for during a considerable period. For this reason most breeds are quite constant for earlobe color.

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Although the earlobe is one of the conspicuous furnishings of the head of the chicken, we find little mention of it in genetic literature. Numerous crosses have been made involving differences in earlobe color but practically nothing has been written upon the inheritance of its color variations. The variability of the F_1 generation and the complex nature of its factorial basis have probably been the conditions which have discouraged the undertaking of genetic investigations of this character.

This study is an attmpt to analyze as completely as our knowledge of the genetics of poultry will permit, the factorial basis of earlobe color.

BREEDS USED

Most of the conclusions of this paper are based upon the results of crosses of the Jersey Black Giant and the Single Comb White Leghorn breeds. However, in the course of other genetic studies, crosses of several breeds differing in earlobe color have been made and in all cases accurate records have been kept. The white-earlobe breeds used in this study were the Single Comb White Leghorn, Rose Comb Brown Leghorn, and Single Comb Buff Leghorn. The red-earlobe breeds were Jersey Black Giant, Barred Plymouth Rock, Single Comb Rhode Island Red, Dark Brahma, White Wyandotte and Silver Laced Wyandotte. The tables presented in the following discussion will indicate the crosses made of the various breeds.

CLASSIFICATION OF EARLOBE COLOR

Records of earlobe color were made at three months and six months of age. It was found that the description for the three-month age was not entirely reliable since changes in color sometimes took place afterwards. The description at the age of six months was used for classification since color attained at that age appeared to be the normal adult color. Any changes after this age were usually due to injury. The fact that only those individuals raised to the adult condition could be classified, accounts for the small numbers in some crosses.

The resulting offspring exhibited all gradations between entirely red and entirely white earlobes. For purposes of classification it became necessary to establish arbitrary groupings of the intermediate colors. Four grades were recognized; namely, white, predominately white, predominately red, and red. It is realized that there is some experimental error in using so crude a system of classification, but since it was the more accurately defined first and last grades that were of primary interest it seemed to satisfy the needs of the experiment at hand. The intermediate grades were not cases of blending of the red and white but the mingling

of the two in varying proportions. The variations are probably quantitative ones depending upon the amount of white tissue deposited and the relative amounts of white and red are determined by the proportion of the surface of the earlobe covered by the white.

F_1 GENERATION

For study of the results of mating red and white earlobe breeds of poultry, data were available from ten different crosses. In table 1 are

TABLE 1

Distribution of earlobe color for the F_1 generation of various crosses.

Mating	Females				Males			
	White	Predominately white	Predominately red	Red	White	Predominately white	Predominately red	Red
RED EARLOBE MALES BY WHITE EARLOBE FEMALES								
1 Black Giant male by White Leghorn female	1	20	27	2	2	17	24	1
2 Rhode Island Red male by White Leghorn female		2	21	1	1	1	19	3
3 Dark Brahma male by Brown Leghorn female	3	7	6	1	2	3	7	5
4 Barred Rock male by Brown Leghorn female	1	1	5	3	1	1		2
5 Barred Rock male by Buff Leghorn female	1	5	9	1	1	9	9	7
6 White Wyandotte male by Buff Leghorn female	6	2	1		1	7	1	
WHITE EARLOBE MALES BY RED EARLOBE FEMALES								
7 White Leghorn male by Black Giant female	12	11	6	1	12	11	7	
8 Brown Leghorn male by Silver Laced Wyandotte female.	3	4	5		3	3	5	2
9 Brown Leghorn male by Silver Pencilled Rock female	6	6	1		2	11	7	
10 White Leghorn male by Rhode Island Red female		9	19	3			14	9

shown the results of these crosses, six of which are matings of red earlobe males by white earlobe females and four in which the reciprocal relations were true. In two crosses, Jersey Black Giant by White Leghorn and Rhode Island Red by White Leghorn, reciprocal crosses of the same mating were available. A glance at table 1 will show that complete dominance of either color was not obtained. Also it is seen that the different matings of the same type (involving different breeds) are somewhat variable with respect to the proportions of red, white and intermediate colors. The small number of individuals involved in some matings may be responsible for the variability but later generations indicate that the various breeds may differ as to their genetic constitution for genes affecting earlobe color.

Variability of the F_1 Generation

In most crosses it is seen that all four grades of earlobe color are found in the F_1 generation. It is of interest to determine whether these variations are due to variability in dominace or lack of homozygosity. Either or both conditions may contribute to the variability of the F_1 individuals. Since a large number of F_1 individuals were not mated, only a limited amount of data are available for determining whether F_1 individuals differing in earlobe color actually differ in genetic constitution. From the cross of Jersey Black Giant by White Leghorn three F_1 females were mated to the same male. One of these females had red earlobes while the earlobes of the other two were predominately white. Only female offspring are listed here since it will later be shown that sex-linked factors are also involved in this cross. The ratios of the earlobe colors of the female offspring were as follows:

Hen Number	*White*	*Predominately white*	*Predominately red*	*Red*
5388–Red Earlobe		7	6	1
5415–Predominately White	3	3	1	
5452–Predominately White	3	9	2	

It will be seen that the offspring differ in accordance with differences among the F_1 parents.

Two F_1 males were backcrossed to several Jersey Black Giant females. One having predominately red earlobes produced female offspring which were classified as follows; 14 predominately white; 54 predominately red and 28 red. The other which had predominately white earlobes gave the following distribution of earlobe colors; 10 predominately white, 23 predominately red and 7 red. Here again the distribution of color among the offspring differed in accordance with the difference between the two F_1 males.

In the cross of Brown Leghorn by Silver Penciled Rocks two F_1 males were used. They were full brothers but one had earlobes classified as white and the other as predominately white. For certain other genetic studies, the two males were each mated to F_1 females from cross of Brown Leghorn by Buff Leghorn and Brown Leghorn by Rhode Island Red. In table 2 the results of these matings are shown. Male 987M which had

TABLE 2

Variability of earlobe color of F_1 individuals. Results of mating two F_1 males differing in earlobe color to similar lots of females; showing that the variability in the F_1 generation is due to differences in their genetic constitution.

Male Number	Females				Males			
	White	Predominately white	Predominately red	Red	White	Predominately white	Predominately red	Red
MATED TO F_1 FEMALES FROM BROWN LEGHORN—BUFF LEGHORN CROSS								
987M (white earlobe)	53				42			
989M (predominately white)	21	3	1		52	3		
MATED TO F_1 FEMALES FROM BROWN LEGHORN—RHODE ISLAND RED CROSS								
987M	38	9	2	1	31	3		
989M	21	16	4	2	20	9	3	

the entirely white earlobes produced offspring whose earlobes showed a greater amount of white than did the progeny of 989M. In the first mating shown in table 2 the autosomal constitution of the offspring was such that most of the earlobes were white but the few which were not entirely white came from male 989 M. In the second mating more earlobes appeared which showed varying degrees of red and here the offspring of 989 M had earlobes which averaged considerably more red. Since these two males were full brothers the difference in their genetic constitution with respect to factors influencing earlobe color must have been due to heterozygosity for these factors on the part of one or both parents.

The foregoing data seem to indicate that the offspring of F_1 individuals varying in earlobe color, tend to vary in the same direction as their parents. This may be due to the fact that the parental stock was not entirely homozygous for the factors involved and that we are dealing with a multiple factor situation. Since the breeds used seldom showed by their own variability any indication of heterozygosity it would be

difficult to establish homozygosity before crossing. These factors although exerting no apparent influence in the pure breed, did appear to be effective when the other factors were in a heterozygous condition.

SEX-LINKED FACTORS

Reciprocal F_1 matings of the same cross may be utilized to determine whether factors influencing earlobe color are carried by the sex-chromosome. In two crosses, White Leghorn by Jersey Black Giant and White Leghorn by Rhode Island Red, data from reciprocal crosses are available for comparison. Variability of the genetic constitution of individuals of a breed is here a source of error but it is believed that individuals of a single strain do not differ greatly in their constitution with respect to the major factors for earlobe color. If sex-linked factors affecting earlobe color are operative we would expect the female offspring of reciprocal crosses to differ. Since in the chicken, the male is the homogametic and the female the heterogametic sex, the female offspring of any cross receive their only Z- or sex-chromosome from the father. Since the female has only one Z-chromosome she should exhibit all sex-linked characters of her father. Thus if there are sex-linked factors affecting earlobe color, females from reciprocal F_1 crosses should differ in accordance with the effects of these factors in conjunction with those carried by the autosomes when in a heterozygous condition.

Matings 1 and 7 and 2 and 10 of table 1 afford data for comparison of the results of reciprocal F_1 crosses. For the first-mentioned matings the daughters of the white earlobe male show more white in the earlobes than those of the reciprocal mating in which the father had red earlobes. For the second case (Rhode Island Red by White Leghorn) there is only a very slight difference between the reciprocal crosses but such differen ce as exist are in the same direction as the other two reciprocals. If these differences may be taken as significant they would seem to indicate that some determiners for earlobe color are located in the Z-chromosome. It should be noted, however, that in the cross of Jersey Black Giant by White Leghorn the male offspring of reciprocal crosses differ in the same manner as do the females. Since all sons receive a Z-chromosome from each parent, the male offspring of reciprocal crosses should not differ with respect to the sex-linked factors. So from the F_1 generation no conclusions can be drawn regarding the existence of sex-linked factors affecting earlobe color. From the distribution of F_1 males it cannot be said that either color of earlobe is dominant. Most of the F_1 individuals have earlobes which are intermediate in color.

The results of various crosses of the White Leghorn and Jersey Black Giant are given in table 3 for the purpose of showing whether important

TABLE 3

Results of the various crosses of White Leghorns and Jersey Black Giants for the study of inheritance of earlobe color.

SIRE	DAM	SONS	DAUGHTERS	WHITE	PREDOMINATELY WHITE	PREDOMINATELY RED	RED
1 Giant ***RR****RR*	Leghorn ***WW****W*	***RW****RW*	***RW****R*	♀ 1 ♂ 2	20 17	27 24	2 1
2 Leghorn ***WW****WW*	Giant ***RR****R*	***RW****RW*	***RW****W*	♀12 ♂12	11 11	6 7	1
3 F_1 ***RW****RW*	F_1 (G♂×L♀) ***RW****R*	***RR*** *RR* ***RW*** *RW* ***WW*** *RW*	***RR*** *R* ***RW*** *W* ***WW*** *W*	♀10 ♂ 2	27 28	17 36	1 10
4 F_1 ***RW****RW*	F_1 (L♂×G♀) ***RW****W*	***RR*** *RW* ***RW*** *WW* ***WW*** *WW*	***RR*** *R* ***RW*** *W* ***WW*** *W*	♀16 ♂30	51 42	34 28	10 8
5 F_1 ***RW****RW*	Giant ***RR****R*	***RR*** *RR* ***RW*** *RW*	***RR*** *R* ***RW*** *W*	♀.. ♂..	14 3	54 29	28 56
6 F_1 ***RW****RW*	Leghorn ***WW****W*	***RW*** *RW* ***WW*** *WW*	***RW*** *R* ***WW*** *W*	♀17 ♂22	10 2	1 1	.. ..
7 Leghorn ***WW****WW*	F_1 (G♂×L♀) ***RW****R*	***RW*** *RW* ***WW*** *RW*	***RW*** *W* ***WW*** *W*	♀31 ♂33	1 3	.. 3	.. ..

sex-linked factors are involved in the determination of earlobe color. Because of the variety of F_2 matings made in the cross this set of data is most satisfactory for analyzing the situation. In this table symbols are used to indicate the genetic constitution of each parent and the male and female offspring. The symbols in bold-face type indicate the origin of the two members of any pair of autosomes which may carry major factors influencing earlobe color. The symbols in italics show the origin of the Z-chromosome. The letter W in bold-face type signifies that the particular autosome was derived from the white earlobe parent while the same letter in italics marks a Z-chromosome originating in the white earlobe parent. The letter R is used in the same manner to indicate chromosomes originating in the red earlobe parent. Under the son and daughter headings are found listed all possible genotypes with reference to the sex chromosomes and any one pair of autosomes.

The histograms of figure 1 show graphically the distribution of earlobe color for both males and females of the last five matings of table 3. The solid line represents the distribution for the females and the dash line for the males. The four sections of each histogram represent the four grades of earlobe color; namely, white, predominately white, predominately red and red. The left hand section of each histogram is for white and the other

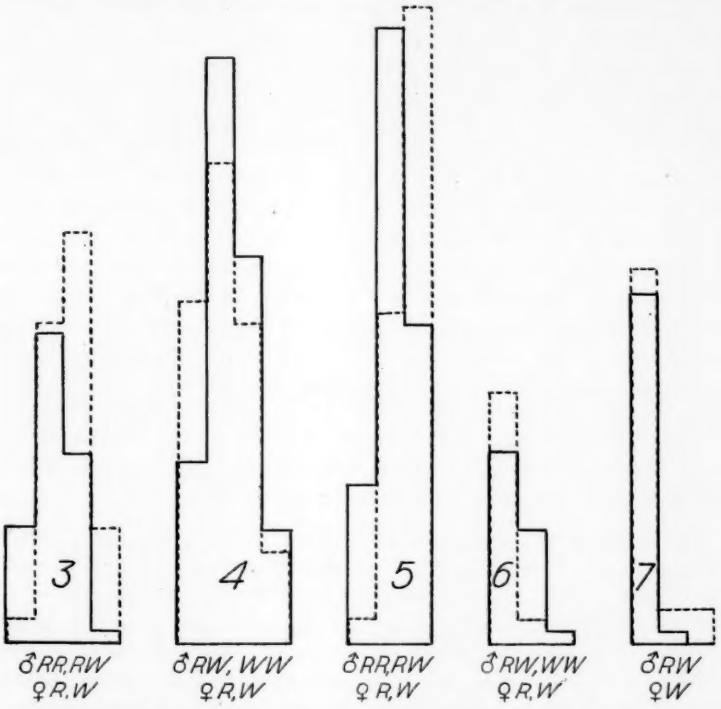

FIGURE 1.—The effect of sex-linked determiners upon earlobe color. The histograms present graphically a comparison of the distribution of earlobe color for males and females of the last five matings in table 3. The solid line indicates the distribution for females and the dash line for males. The letters below the graphs indicate the origin of the sex-chromosomes for the two sexes, *R* being for chromosomes originating in the red earlobe stock and *W* for those from the white earlobe stock.

grades follow in the order just given. Below each diagram is indicated the origin of the Z-chromosomes in the two sexes. In each mating, the genetic constitution of males and females with respect to autosomal genes is, of

course, the same. If differences between the two sexes are consistently associated with differences in origin of the sex chromosomes then it may be held that they are due to varying constitutions of the individuals from which the chromosomes came.

For all matings except the last it will be seen that the females are identical with respect to their Z-chromosomes, half receiving their only sex chromosome from the red and half from the white earlobe breed. In the histogram for mating 3 it will be seen that the earlobes of the males vary more in the red direction than do those of the females. Here half of the males are homozygous for sex chromosomes from the red earlobe stock while the other half are heterozygous. The two sexes are identical with respect to their autosomal constitution so if we assume that the diploid condition as found in the male is more effective than the haploid in the female, then the males should vary more in the red direction than do the females. In mating 4 it will be noted that the males are equally divided between those homozygous for the "white" chromosome and those heterozygous for the sex-linked factors. If the factors carried by the sex chromosome influence earlobe color, then it would be expected that the males would have whiter earlobes than those in the previous mating(since the autosomal constitution of the two matings are the same) and that they should be whiter than those of their sisters. An examination of histograms 3 and 4 will prove that both these conditions are met. The females of these two matings are identical with respect to both sex chromosomes and autosomes and their histograms also are quite similar.

The histograms 5 and 6 are for matings of the same designation in table 3. It will be seen that since in mating 5 the males are half homozygous for the "red" chromosome and half heterozygous they should have earlobes which are less white than their sisters. In mating 6, the formulas of their sex chromosomes would indicate that the males should have whiter earlobes than their sisters. The histograms for these two matings show the distribution of color of earlobes for the two sexes to be in agreement with their sex chromosomal composition.

The last histogram which represents the distribution of earlobe color for mating 7 shows practically no difference between the color of earlobes of the two sexes. It will be noted that the males are all heterozygous for any factors on the sex chromosome influencing earlobe color. This result is in agreement with the reciprocal F_1 matings. These two matings indicated in table 3 as 1 and 2 show the distribution of earlobe color to be quite similar for the two sexes.

The results in table 3 and figure 1 show fairly clearly that sex-linked factors are operative in influencing earlobe color. It would seem, however, that the determiners for earlobe color carried by the Z-chromosome are effective only when they are in a homozygous and diploid condition. It is true, at least, that in all matings where half of the male offspring were homozygous for genes of the red or white earlobe stock they differed from the females in earlobe color in the expected direction. In matings where the males were all heterozygous for sex-linked determiners there was no difference between the earlobe color of the males and females.

In crosses of other breeds the matings are not as complete as the one involving Jersey Black Giants and White Leghorns. The matings of Rhode Island Reds and White Leghorns shown in table 4 indicate that

TABLE 4

Results of crosses of White Leghorn by Rhode Island Red.

SIRE	DAM	SONS	DAUGHTERS	WHITE	PREDOMINATELY WHITE	PREDOMINATELY RED	RED
Red ***RRRR***	Leghorn ***WWW***	***RWRW***	***RWR***	♀ .. ♂ 1	2 1	21 19	1 3
Leghorn ***WWWW***	Red ***RRR***	***RWRW***	***RWW***	♀ .. ♂ ..	9 ..	19 14	3 9
F_1 ***RWRW***	F_1 (*Rd*♂ × *L*♀) ***RWR***	***WW*** ***WR*** *RW* ***RR*** *RR*	***WW*** ***WR*** *R* ***RR*** *W*	♀ 5 ♂ 3	12 13	22 23	7 7
F_1 ***RWRW***	F_1 (*L*♂ × *Rd*♀) ***RWW***	***WW*** ***WR*** *RW* ***RR*** *WW*	***WW*** ***WR*** *R* ***RR*** *W*	♀ 2 ♂ 5	4 9	16 15	6 11

sex-linked factors are not operative. An examination of these matings shows that although the sons of the two F_2 matings differ in their sex chromosomes, their earlobe colors are quite similar and differ but little from those of their sisters.

In table 5 are shown the F_2 results from crosses of Brown Leghorn by Silver Penciled Rocks. Here the white earlobe is carried by the Brown Leghorns and the red by the Penciled Rock. A comparison of the distribution of earlobe color and the sex-chromosomes indicates slightly that sex-linked factors may be involved but evidence supporting this view is not found in all matings.

TABLE 5

Results of crosses of Brown Leghorns by Silver Penciled Rocks.

SIRE	DAM	SONS	DAUGHTERS	WHITE	PREDOMINATELY WHITE	PREDOMINATELY RED	RED
F_1	$F_1(L\sigma \times Rd\,♀)$	WW, RW → RW; RR → WW	WW, RW → R; RR → W	♀21	26	14	1
RWRW	RWW			♂32	26	5	1
F_1	Br. Leghorn	RW → RW; WW → WW	RW → R; WW → W	♀59	4	2	..
RWRW	WWW			♂59	1	..	..
F_1	S. P. Rocks	RW → RW; RR → RR	RW → R; RR → W	♀ 2	2	4	1
RWRW	RRR			♂ 1	1	2	4

Table 6 gives the data for the F_2 results of mating Buff Leghorn by

TABLE 6

Results of crosses of Buff Leghorn by White Wyandotte and Barred Plymouth Rock.

SIRE	DAM	SONS	DAUGHTERS	WHITE	PREDOMINATELY WHITE	PREDOMINATELY RED	RED
WYANDOTTE×BUFF LEGHORN							
F_1	$F_1(Wy\sigma \times L\,♀)$	WW, RW → RW; RR → RR	WW, RW → R; RR → W	♀85	15	3	
RWRW	RWR			♂40	38	5	3
F_1	W. Wyandotte	RW → RW; RR → RR	RW → R; RR → W	♀ 2	9	12	5
RWRW	RRR			♂	1	8	13
BARRED ROCK×BUFF LEGHORN							
F_1	$F_1(Rk\sigma \times L\,♀)$	WW, RW → RW; RR → RR	WW, RW → R; RR → W	♀14	15	20	15
RWRW	RWR			♂12	12	11	9
F_1	Buff Leghorns	WW, RW → RW; RW → WW	WW, RW → R; RR → W	♀42	12	5	1
RWRW	WWW			♂39	13	4	2

White Wyandotte and Buff Leghorn by Barred Plymouth Rock. In the former cross there is evidence that sex-linked factors are involved in bringing about the difference in earlobe color. The data shown for the Buff Leghorn-Barred Plymouth Rock cross, if checked for the correspondence in distribution of sex chromosomes and earlobe color, furnish no evidence for the view that sex-linked factors are here operative.

AUTOSOMAL FACTORS

The data already presented indicate that sex-linked factors are involved in determining earlobe color in some breeds. In some cases their influence appeared to be slight and in other crosses there was no evidence for sex-linkage of factors responsible for the existing differences in earlobe color. These results would lead one to expect evidence for autosomal factors for color of the earlobe. If so the data already presented in table 3 should afford such evidence. The columns giving the genotypes of the male and female offspring show some instances where the constitution with respect to the sex chromosome are identical but where differences in the autosomal constitution exist. The female offspring of matings 4 and 5 offer material for a comparison of this sort. Although their sex chromosomal constitution is the same we find a wide difference in their earlobe color. Associated with this difference in distribution of color of earlobes we see that there is a difference in the autosomal constitution of the two lots of females. Those in mating 4 have the three genotypes which result from mating two heterozygous individuals, one-fourth homozygous for white, one-half heterozygous, and one-fourth homozygous for red. In mating 5, half the females are homozygous for red and half heterozygous, none being homozygous for white. The great difference found here shows that the major factors involved are probably autosomal in location.

A still greater difference is shown by matings 5 and 6. Here the female offspring of the two matings have identical sex-chromosomal composition but differ considerably in the origin of their autosomes. In 5 half are homozygous for factors of the autosomes from the red earlobe parent while in 6 half are homozygous for factors from the white earlobe stock. In each case half of the offspring are also heterozygous. Since mating 6 produces no phenotypes homozygous for the R-factor we would expect it to differ from mating 5 to a greater degree than does mating 4. These relations are in agreement with differences in the factorial constitution of their autosomes. Matings 5 and 6 are reciprocal back crosses to the parent stocks and since in mating 6 a higher percentage of whites occur than reds in mating 5 there is evidence that at least some of the white autosomal factors are partially dominant to the red.

The evidence is fairly conclusive that at least one pair of autosomal factors is effective in determining earlobe color. A comparison of the results of matings 2 and 7 seems to indicate that more than one pair of autosomal factors are operative. The female offspring of the two matings have identical constitution with respect to the sex chromosomes. They

differ in that in mating 2 all are heterozygous for autosomal factors while in mating 7 half are of this constitution and half are homozygous for any given pair of autosomes from the white earlobe parent. If only one pair of autosomes are involved in determining earlobe color, then half of the female offspring in mating 7 should have earlobes the color of which show a distribution similar to that in mating 2. The data show that practically all of the females in mating 7 have white earlobes. Most of the expected intermediates are missing. This shortage of intermediates might be expected if there exists another autosomal factor which segregates independently of the first. If either autosomal factor in a homozygous condition is capable of producing birds with white earlobes but few earlobes other than white would be expected. It thus seems probably that more than one pair of autosomal factors are operative.

LINKAGE OF SEX-LINKED DETERMINERS

Since it has been shown that both autosomal and sex-linked factors are involved in determining earlobe color it is of interest to know whether earlobe color shows linkage with other known characters. The fact that some of its determiners show sex-linked behavior suggests that earlobe color should show linkage with some member of the sex-linked group. Since something is known of the distribution of the sex-linked factors it is possible to determine with some degree of accuracy the relative position of at least the major sex-linked factors influencing earlobe color. The writer (Anatomical Record **29**: 143–144) has shown that the factors for the sex-linked character determining rate of feathering is widely separated on the sex chromosome from those for barring and shank color. Hence, a cross involving these characters and differences in earlobe color should show in which end of the sex chromosome the sex-linked factor for earlobe color lies.

In table 7 are shown the linkage relations of earlobe color with rate of feathering and shank color, and rate of feathering and barring. These data are from the F_2 generation of crosses of the Jersey Black Giant and White Leghorn. In the table are included only the crossover classes, first for rate of feathering and shank color, and then rate of feathering and barring. Since the original combinations were rapid feathering and yellow shanks, and slow feathering and dark shanks, the crossover classes in the first section of the table are slow feathering-yellow and rapid feathering-dark. In the original crosses white earlobe entered with yellow shanks and rapid feathering. Since the determiners for yellow and rapid have been shown to occupy distant sections of the sex chromosome, the crossover

classes in table 7, representing breaks in the sex chromosome between these points should indicate the position of the sex-linked determiners for earlobe color in relation to these two factors. When the determiners for yellow and rapid separate in crossing over, the determiners for white earlobe will go most frequently with the point with which it is most closely associated. Thus, since the original combination was yellow shank and rapid feathering, the two crossover classes will include each character combined with the allelomorph of the other and the group having the whiter earlobes will show which of these two points the earlobe color factor lies nearer.

Figure 2A shows the probable relation upon the sex chromosome of factors for rate of feathering and shank color. The diagrams 2B and 2C show the sex chromosome following the phenomenon of crossing over,

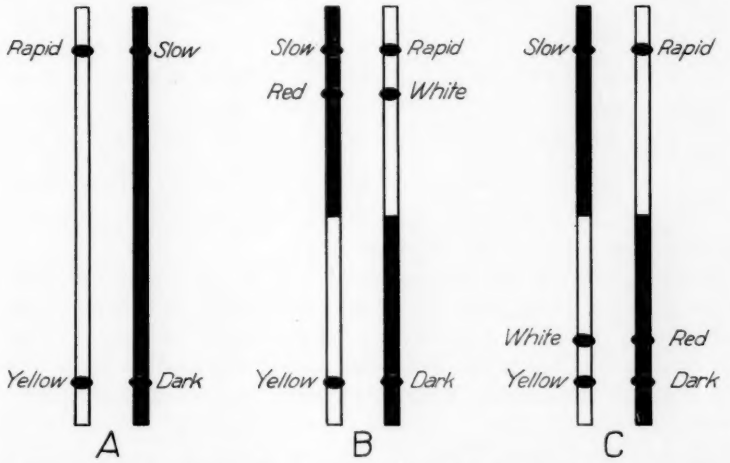

FIGURE 2.—Diagrams showing the various crossover combinations depending upon which end of the chromosome the sex-linked factor for earlobe color is located. Figure 2A gives the probable location of the factors for rate of feathering and shank color. Figure 2B gives the most frequent crossover combinations when the earlobe color factor is nearer the gene for rate of feathering while figure 2C shows the situation where it is located nearer the gene for shank color. In table 7 are found the data for these linkage relations.

B indicating the most frequent grouping of characters providing the gene influencing earlobe color is in the same end with that for rate of feathering. The situation when the determiner for earlobe color is in the opposite end is shown in C. If the situation shown in B be the true one,

then the crossover group rapid-dark should be whiter than its reciprocal slow-yellow. If the diagram C represents the true relation then the conditions stated above would be reversed, the crossover group slow-yellow being the whiter.

Table 7 shows the crossover group slow-yellow to be whiter than its reciprocal and hence substantiates the view that the sex-linked determiner

TABLE 7

Linkage relations of sex-linked factors for earlobe color.

Crossover group	Earlobe color			
	White	Predominately white	Predominately red	Red
RELATION TO RATE OF FEATHERING AND SHANK COLOR				
Rapid dark	4	17	13	5
Slow yellow	16	22	11	4
RELATION TO RATE OF FEATHERING AND BARRING				
Rapid non-barred		4	9	4
Slow barred	1	3	3	3

for earlobe color is located in the chromosome nearer the shank color determiner than that for rate of feathering.

It has also been shown (WARREN 1924) that the determiner for barring is also distantly separated from the rate of feathering gene and probably located near the gene for shank color. If this be true then earlobe color should show relations to this factor similar to those found for shank color. The original combination was white earlobe, rapid feathering, barred plumage so the barred-slow crossover group should be whiter than the group rapid-non-barred. There is a slight difference in distribution in the expected direction. Since the numbers involved are small these results cannot be held to be conclusive but may be taken as a suggestion of the possible relations. Also, since the effects of the sex-linked determiners have been shown to be slight in the presence of the autosomal factors, large differences could not be expected in the data shown in table 7.

LINKAGE OF AUTOSOMAL FACTORS

It is also possible to determine whether linkage relations exist between any autosomal earlobe color factors and factors for certain known autosomal characters. Several autosomal characters were involved in the

crosses, but for only two have the linkage been measured since in most instances the characters were not sufficiently clean-cut in their segregation to permit accurate classification. No autosomal linkage groups have yet been determined in poultry so it is not known whether the characters used belong to the same or different linkage groups. Although dominant white and extended black plumage colors have been treated as an allelomorphic pair the evidence substantiating this assumption is not entirely conclusive. This fact should not however influence the results.

Any character, the factor for which lies in the same chromosome with autosomal earlobe factors, should in segregation more frequently be associated with the earlobe color with which it originally entered. Thus in a cross involving white and black plumage and red and white earl obes, since white plumage and white earlobe entered the cross together, it is expected that in the F_2 generation the white plumage segregates will have the whiter earlobes, providing the factors for these two characters are in the same chromosome. The segregation of rose and single comb should in the same manner indicate their linkage relations with earlobe color. If no linkage exists the distribution of earlobe color among the classes of segregates should be a random one. Table 8 shows the distribution of

TABLE 8

Linkage relations of autosomal factors for earlobe color.

CONTRASTED PAIR	EARLOBE COLOR			
	White	Predominately white	Predominately red	Red
White		9	46	44
Black		8	35	35
Rose	67	56	16	2
Single	29	21	9	1

earlobe color for F_2 segregates of black and white plumage and rose and single comb. It will be noted that the distribution of earlobe color for the two members of each group is practically identical, thus indicating that the determiners for these characters are not carried by any chromosomes bearing the major autosomal determiners for earlobe color.

LINKAGE RELATIONS OF EARLOBE COLOR AND EGG COLOR

In the established breeds and varieties of poultry white egg shell color has usually been associated with white earlobe color, while the red earlobe breeds usually lay eggs of the darker shades. This has led practical breeders

to hold that there is some relation between earlobe and egg color. The apparent linkage might be due to close association of the determining genes on the chromosome or to the existence of a single factor which controls the pigmentation of both the egg and earlobe. If the existing association between earlobe and egg color is a chance one, the linkage should be broken by segregation.

To determine the nature of the association between these two characters 62 F_2 females chosen at random from the cross Jersey Black Giant by White Leghorn were tested for egg color. For classifying (the egg colors) a color chart was made grading from white to the darkest brown shade. The color grade was determined by taking the average color of five eggs laid during the first month of production. Other studies have shown that egg color varies somewhat with the period of production and for that reason the selection was limited to the first month.

In table 9 is shown the distribution of egg color for the 62 females,

TABLE 9

Showing independent assortment of factors for earlobe color and egg shell color.

EARLOBE COLOR	EGG COLOR GRADES								
	1	2	3	4	5	6	7	8	9
White	..	..	1	1	4	..	1	1	..
Predominately white	2	2	2	5	8	5	1	..	1
Predominately red	..	1	4	4	6	4	2	..	..
Red	2	1	..	1	2	1	1	..	..

grouped according to earlobe color. Grade 1 is white and the succeeding grades become progressively darker. It is seen that individuals falling into the four grades of earlobe color lay eggs of practically the same average color and hence there is no evidence for any conditions which tend to associate white earlobe with white egg color. This would seem to show that the existing association is entirely a chance one. It is possible that this association may bear some relation to the origin of breeds of poultry.

GENERAL DISCUSSION

The experiments discussed indicate that earlobe color is dependent upon several genetic factors. The fact that all grades of earlobe color are found in the F_1 generation from crossing most white and red earlobe breeds would lead one to expect a complex situation.

It also seems probable that individuals of a single strain of a breed may differ in some of the minor factors influencing earlobe color. The results of the F_2 generation indicated that individuals of some breeds were not homozygous for all factors for earlobe color. Greater differences in factorial constitution probably exist between breeds. Crosses of the Rhode Island Red breed showed no evidence of its carrying sex-linked earlobe color determiners while crosses of other breeds indicated genes of this chromosome to be operative. The differences in genetic constitution of individuals of a breed do not seem to affect the major earlobe color factors since the breeds dealt with seldom produced individuals varying from the standard.

From crosses of the Single Comb White Leghorn and Jersey Black Giant breeds there is evidence for the existence of at least three factors influencing earlobe color. A sex-linked factor is operative but seems to play a minor role since its influence was slight and exhibited only when in a homozygous diploid condition and acting with some of the autosomal factors in a heterozygous condition. The major earlobe color factors are autosomal and there is evidence that at least two factors of this group are operative. This complex situation is probably only the natural result of the manner in which the color has been fixed. Since the slightest variation from either white or red constitute a breed disqualification any modifying factor which tended to maintain the constancy of the desired color was seized upon and by selection incorporated in the genetic constitution of the breed.

SUMMARY

1. Earlobe color has a complex factorial basis.

2. Breeds having the same earlobe color may differ considerably in their genetic constitution with respect to this character.

3. Individuals of a single breed and strain may differ in some of the minor genetic determiners.

4. The Jersey Black Giant and White Leghorn breeds differ in at least three factors determining earlobe color, one sex-linked and two autosomal.

5. It was possible to estimate roughly the position upon the sex chromosome of the sex-linked factor.

6. The autosomal factors for earlobe color showed no linkage with those for any autosomal characters which the crosses permitted testing.

7. There was no evidence of any linkage between the factors for earlobe color and egg color.

A NEW SEX-RATIO ABNORMALITY IN *DROSOPHILA OBSCURA**

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MATERIALS AND METHODS

Drosophila obscura Fallén is commonly found in the neighbourhood of Moscow. Apparently, it in no way differs from the American fly; at least, the coincidence between all the morphological characters of both species is complete. The cytological investigation of the Russian *D. obscura* is unfinished as yet; some preliminary data, which S. L. FROLOVA most obligingly communicated to me, mark the presence of a long V-shaped X-chromosome, perfectly distinct from the rodlike Y-chromosome. The American *D. obscura*, according to METZ and MOSES (1923), has a bent X-chromosome which, as in the Russian species, differs distinctly from the straight and shorter Y-chromosome.

In America *D. obscura* is usually raised upon bananas; in our laboratory, in spite of many experiments, no completely satisfactory diet has yet been found. The best results are obtained with a nutritive medium prepared from a mixture of fermented raisins (100 g.), potatoes (400 g.), agar-agar (4 g.) and water (800 ccm.), but even on this medium the percent of perishing cultures is comparatively large. Also the number of offspring produced by one pair is less than that usually observed in other species of Drosophila. I could never succeed in obtaining a mating that produced more than 200 flies, and the mean number for the offspring in a culture did not rise above 40–45.

* The GALTON AND MENDEL MEMORIAL FUND pays part of the cost of the accompanying tables.

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The flies were raised in sterilized flat-bottomed glass cylinders, containing some nutritive medium lightly sprinkled with yeast; the cylinders were corked by thick cotton-wool stoppers. The cultures were kept in a temperature oscillating from 21° to 25°C. Particular attention was paid to the virginity of the females used for mating; only such were considered virgin as had yet folded wings, or had been hatched in the absence of males (for instance from isolated pupae). On an average, the development of the fly took about 20 days, but this term varied considerably, chiefly in accordance with the temperature. The flies hatched were narcotized with ether and carefully examined through a binocular microscope, all the results being recorded in detail.

TABLE 1

OFFSPRING MORE THAN 20 FLIES

NUMBER OF CULTURES	NUMBER	♂♂	♀♀	TOTAL	PERCENT ♂♂
	1	21	23	44	47.7
	3	14	16	30	46.7
	4	13	13	26	50.0
	6	26	26	52	50.0
	8	22	18	40	55.0
	9	23	19	42	54.8
	10	20	14	34	58.8
	11	21	21	42	50.0
	12	21	19	40	52.5
	13	10	10	20	50.0
	14	18	25	43	47.9
	15	17	18	35	48.6
	16	14	10	24	58.3
	18	17	15	32	53.1
	19	35	33	68	51.5
	20	13	19	32	40.6
16	..	305	299	604	50.4

OFFSPRING LESS THAN 20 FLIES

NUMBER OF CULTURES	..	♂♂	♀♀	TOTAL	PERCENT ♂♂
3	..	20	28	48	41.7

TOTAL

NUMBER OF CULTURES	..	♂♂	♀♀	TOTAL	PERCENT ♂♂
19	..	325	327	652	49.9

APPEARANCE OF THE ABNORMALITY

In July, 1925, I caught in a forest near the Hydrophysiological Station in Zvenigorod (near Moscow) 19 females of *D. obscura*. They were all fecundated already before being caught, and the offspring they gave were perfectly normal as regards the sex-ratio (table 1). Altogether 327 females and 325 males were obtained, thus giving a relation of 1.003:0.997, instead of the expected 1:1. From each culture of the first generation ten pairs of flies were inbred. In the F_2, both in the sum total and in the separate crosses the offspring of 17 of the wild females showed a normal sex-ratio. At the same time in the progeny of two females a sharp deviation from the expected relations was found. The appearance of these abnormalities is shown in figure 1. In two lines, line 3 and line 13, cultures

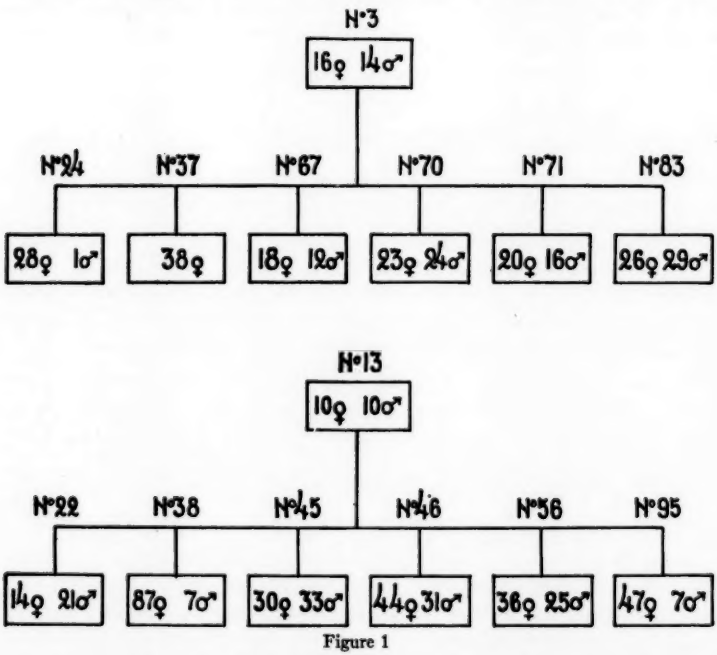

Figure 1

appeared totally or almost totally consisting of females. Altogether, four such cultures were obtained (Nos. 24 and 37 in line III and Nos. 38 and 95 in line XIII). The deviations from the normal sex-ratio were so considerable that it seemed impossible to explain them by accidental causes. A further analysis of the phenomena was therefore undertaken.

CHARACTERISTICS OF THE ABNORMALITY

The females resulting from the abnormal cultures of the second generation of lines 3 and 13 were mated in both lines partly with their brothers, partly with males from kindred normal cultures. The first generation proceeding from these matings showed itself everywhere perfectly normal. Further, in the succeeding generations, the offspring of these flies were bred in both lines by close inbreeding as well as more freely, within the limits of the line. A part of these crosses always showed a normal sex-ratio, while the other gave a progeny resembling, as to its results, the original abnormal cultures, namely, either consisting exclusively of females, or presenting only an insignificant number of males. Both in lines 3 and 13 the number of males in these cultures varied between 0 and 15 percent, on an average however not exceeding 4 to 5 percent of the total number of flies. Henceforth cultures showing such a predominance of females will be spoken of as "female" ones.

For several consecutive generations no more detailed investigation of the described abnormality was undertaken. During all this period I confined myself to maintaining line 3 by crossing the females from the "female" cultures either with their brothers, if such existed, or else with males from normal cultures of the same line. Also the progeny of these normal cultures was occasionally inbred. In all these cases "female" cultures were obtained as well as normal ones. As a result of all these crossings I was able to collect somewhat extensive statistical materials, which showed in line 3 of *D. obscura* the presence of but two types of sex-ratios, the normal and the "female" one, and the total lack of any transitions between them. The results are represented in figure 2. They include all the individual matings, in which the flies of line 3 participated, and which resulted in more than 20 offspring. All the matings are divided, according to the percent of males in the offspring, into classes, which are shown on the abscissa. The number of matings belonging to each class are shown on the ordinates. As can be easily seen, all the crosses fall into two independent groups. The first one includes the cultures with a practically normal sex-ratio, that is, with about 50 percent of males. This group constitutes the right curve of the distribution, which is almost regular and shows that the observed slight deviations from the expected relations are due here to accidental causes. All the other cultures form the left curve; the average number of males is here only 4.1 percent of the total number of flies and the mode of the curve lies at zero. No intermediate cultures appeared which could mar this picture.

This table can be compared with another one, constructed exactly on the same plan for the seventeen normal lines of *D. obscura* (figure 3). Figure 3 includes all the individual matings from all the normal lines, that is, from all the inbreeding lines originating from the wild females, except 3 and 13. The distribution of the cultures is here quite analogous

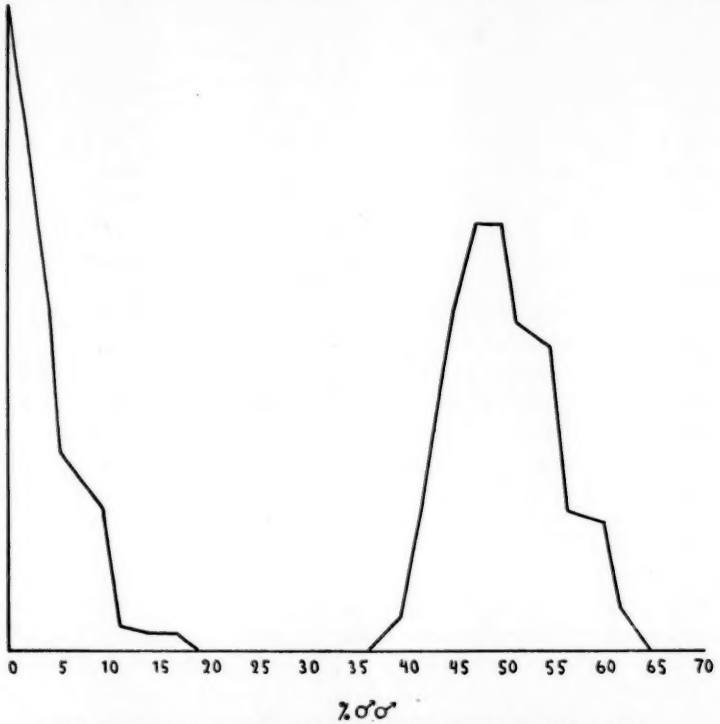

FIGURE 2.—Left curve: M=4.1; n=102 Right curve: M=49.0; n=185.

to that presented by the right curve of figure 2; no "female" or other deviating sex-ratios are met with.

Altogether, the preliminary results of this work can be summed up as follows. The researches upon the seventeen lines, started from wild females, have made it clear that as a rule the deviations from the normal sex-ratio are in *D. obscura* very small and can be easily explained by accidental causes. The crosses in line 3 present at the same time some cultures with abnormal sex relations, the number of males being in them,

on an average, only about 4 percent of the total number of flies. No transitions are observed in this line between the normal and the deviating cultures. An analogous phenomenon was observed in another line (13) while it existed.

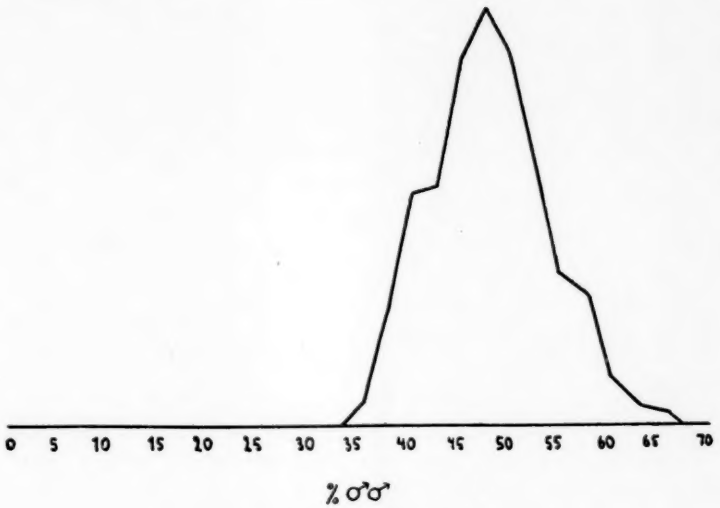

FIGURE 3.—M = 48.0; n = 194.

The data enumerated show that this abnormality is certainly hereditary, but, on the basis of these preliminary investigations it has not been possible to give its more detailed genetical characteristics; in order to do so, a whole new series of special crossings had to be organized.

GENETIC ANALYSIS OF THE ABNORMALITY

By mating a female from the "female" culture No. 1500 with its only brother, an offspring was obtained with a normal sex-ratio (49.4 percent ♂♂) and a comparatively large number of flies. This culture received the No. 1644 and served as a basis for all the subsequent investigations. Two series of crosses were organized from its offspring. The first series, designated as "type I," was produced by individual matings of virgin females from the culture No. 1644 with males of a practically pure normal line of *D. obscura*, that had passed through more than ten generations of close inbreeding without ever presenting any deviations from the usual

sex proportions. All these crosses showed normal sex relations very near 1:1; there were altogether 17 crosses giving more than 20 flies and 5 crosses with a smaller number of offspring. These results are represented in table 2. The second series (type II) consisted of crosses, the reverse of the

TABLE 2

OFFSPRING MORE THAN 20 FLIES

NUMBER OF CULTURES	NUMBER	♂♂	♀♀	TOTAL	PER CENT ♂♂
	1734	10	12	22	45.5
	1735	23	16	39	59.0
	1738	17	15	32	53.1
	1742	21	21	42	50.0
	1744	21	14	35	60.0
	1746	26	35	61	42.6
	1751	22	22	44	50.0
	1752	10	11	21	47.6
	1755	21	26	47	44.7
	1758	18	12	30	60.0
	1761	21	23	44	47.7
	1763	17	15	32	53.1
	1765	20	25	45	44.4
	1766	14	19	33	42.4
	1768	24	28	52	46.2
	1770	35	32	67	52.2
	1771	18	15	33	54.5
17	..	338	341	679	49.8

OFFSPRING LESS THAN 20 FLIES

NUMBER OF CULTURES	..	♂♂	♀♀	TOTAL	PER CENT ♂♂
5	..	19	28	47	40.4

TOTAL

NUMBER OF CULTURES	..	♂♂	♀♀	TOTAL	PER CENT ♂♂
22	..	357	369	726	49.2

preceding ones; that is, virgin females from the pure normal line were crossed with males from No. 1644. Without any exception the crosses of this type showed "female" sex-ratios; 27 crosses were obtained, 19 of which gave more than 20 flies (table 3).

TABLE 3

OFFSPRING MORE THAN 20 FLIES

Number of Cultures	Number	♂♂	♀♀	Total	Percent ♂♂
	1673	4	41	45	8.9
	1674	..	24	24	0.0
	1677	2	19	21	9.5
	1680	3	42	45	6.7
	1682	1	32	33	3.0
	1683	1	25	26	3.8
	1685	..	25	25	0.0
	1687	..	34	34	0.0
	1689	..	29	29	0.0
	1690	4	33	37	10.8
	1693	1	26	27	3.7
	1710	2	37	39	5.1
	1713	3	71	74	4.1
	1715	..	51	51	0.0
	1721	..	22	22	0.0
	1726	..	28	28	0.0
	1729	2	28	30	6.7
	1731	..	20	20	0.0
18	..	23	587	610	3.8

OFFSPRING LESS THAN 20 FLIES

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
9	..	2	97	99	2.0

TOTAL

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
27	..	25	684	709	3.5

The offspring of the crosses of type I did not undergo any further analysis. As to type II, two series of crosses were again started from its cultures. One series, named type III, consisted of crosses between virgin females from culture No. 1713 (of type II) and males from the pure normal line. All these crosses gave normal offspring; table 4 presents the numbers obtained. The other group included crosses between normal virgin females and the males out of all those cultures of type II, where they existed in the

TABLE 4

OFFSPRING MORE THAN 20 FLIES

Number of Cultures	Number	♂♂	♀♀	Total	Percent ♂♂
	1820	20	22	42	47.6
	1821	21	16	37	56.8
	1823	30	21	51	58.8
	1826	14	20	34	41.2
	1827	25	24	49	51.0
	1828	48	51	99	48.5
	1829	14	15	29	48.3
	1835	21	25	46	45.2
	1838	15	23	38	39.5
	1840	18	18	36	50.0
	1841	14	17	31	45.2
	1845	26	26	52	50.0
	1846	18	19	37	48.6
	1851	43	52	95	45.3
	1853	16	20	36	44.4
	1854	19	20	39	48.7
	1856	19	16	35	54.3
	1859	21	28	49	42.9
18	..	402	433	835	48.1

OFFSPRING LESS THAN 20 FLIES

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
6	..	25	28	53	47.2

TOTAL

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
24	..	427	461	888	48.1

offspring. Nineteen such crosses (named type IV) were obtained, all of them normal in regard to the sex-ratio (table 5). Lastly, from the offspring of the culture of type III two more types of crossings were organized: the crossings of virgin females from the culture No. 1828 with males of the normal line (type V), and reversed crossings of normal females with males from the cultures of type III (type VI). All the matings of the V type presented normal sex-relations (table 6). On the contrary, in type VI "female" cultures again appeared.

TABLE 5

OFFSPRING MORE THAN 20 FLIES

Number of Cultures	Number	♂♂	♀♀	Total	Percent ♂♂
	1861	14	16	30	46.7
	1865	15	11	26	57.7
	1866	20	25	45	44.4
	1871	24	27	51	47.1
	1873	20	23	43	46.5
	1875	16	22	38	42.1
	1876	12	12	24	50.0
	1880	13	17	30	43.3
	1882	18	18	36	50.0
	1883	34	28	62	54.8
	1885	10	11	21	47.6
	1887	12	10	22	54.5
	1888	29	20	49	59.2
	1890	26	19	45	57.8
	1896	13	14	27	48.1
15	..	276	273	549	50.3

OFFSPRING LESS THAN 20 FLIES

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
4	..	16	25	41	39.0

TOTAL

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
19	..	292	298	590	49.5

The crosses of type VI, though alike in structure, fall into two groups owing to the origin of the participating males: 27 cultures were obtained from the mating of males from the culture No. 1828 with normal virgin females, while 37 cultures resulted from analogous matings of males from culture No. 1851 (also of type III). Both in the first and in the second group "female" cultures appeared, and in both cases very nearly one half of the matings gave a "female" progeny (table 7 and table 8). Altogether 47 matings were obtained (leaving out those which produced less than 20 flies): 23 matings gave a normal offspring, and 24 matings presented typically "female" sex-ratios. Thus the relation between the number of normal matings and of "female" ones was found to be in type VI very near to 1:1 (table 9).

TABLE 6

OFFSPRING MORE THAN 20 FLIES

Number of Cultures	Number	♂♂	♀♀	Total	Percent ♂♂
	2021	10	15	25	40.0
	2023	26	21	47	55.3
	2027	24	17	41	58.5
	2028	19	17	36	52.8
	2032	16	12	28	57.1
	2035	24	30	54	44.4
	2036	11	9	20	55.0
	2037	14	13	27	51.9
	2045	13	12	25	52.0
	2048	19	22	41	46.3
	2051	18	24	42	42.9
	2053	16	13	29	55.2
	2054	18	19	37	48.6
	2060	14	17	31	45.2
	2061	26	26	52	50.0
	2065	10	12	22	45.5
	2066	26	22	48	54.2
17	..	304	301	605	50.2

OFFSPRING LESS THAN 20 FLIES

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
6	..	28	35	63	44.4

TOTAL

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
23	..	332	336	668	49.7

This is the last series of matings undertaken in order to analyse the phenomenon of irregular sex-distribution in *D. obscura*. The examination of the results obtained allows us to draw some important conclusions as to the mode of inheritance of this abnormality.

First of all, wherever the females originate the progeny is perfectly normal, provided they are mated with males belonging to the pure normal line. This is distinctly to be seen in the matings of type I, III and V. Meanwhile, the matings of type II, arranged on the reverse principle,

TABLE 7

OFFSPRING MORE THAN 20 FLIES

Number of Cultures	Number	♂♂	♀♀	Total	Percent ♂♂
	2070	..	34	34	0.0
	2072	23	20	43	53.5
	2073	..	22	22	0.0
	2078	1	41	42	2.4
	2082	19	19	38	50.0
	2084	20	15	35	57.1
	2085	21	28	49	42.9
	2086	12	12	24	50.0
	2089	..	28	28	0.0
	2097	6	74	80	7.5
	2099	3	50	53	5.7
	2102	24	21	45	53.3
	2103	..	43	43	0.0
	2105	26	30	56	46.4
	2106	2	31	33	6.1
	2110	1	31	32	3.1
	2112	26	25	51	51.0
	2117	14	19	33	42.4
	2122	1	19	20	5.0
	2123	30	32	62	48.4
20	..	229	594	823	27.8

OFFSPRING LESS THAN 20 FLIES

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
7	..	25	79	104	24.0

TOTAL

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
27	..	254	673	927	27.4

where the females were taken from the acknowledged normal line, and the males alone belonged to line III, yielded all a clearly expressed "female" offspring; the same was observed for half of the matings of type VI. This indicates that all the males from No. 1644 and half of those from cultures of type III, when mated even with pure normal females, possess the faculty of giving even in the first generation the same deviating sex-ratios as characterize line 3 of *D. obscura*.

TABLE 8

OFFSPRING MORE THAN 20 FLIES

Number of cultures	Number	♂♂	♀♀	Total	Percent ♂♂
	2125	14	14	28	50.0
	2129	4	69	73	5.5
	2131	1	34	35	2.9
	2135	18	20	38	47.3
	2136	..	36	36	0.0
	2138	23	23	46	50.0
	2145	17	11	28	60.7
	2147	1	21	22	4.5
	2148	..	30	30	0.0
	2162	..	44	44	0.0
	2164	20	24	44	45.5
	2165	24	23	47	51.1
	2166	2	41	43	4.7
	2167	..	40	40	0.0
	2174	29	23	52	55.8
	2175	11	13	24	45.8
	2178	24	26	50	48.0
	2179	1	39	40	2.5
	2180	..	20	20	0.0
	2183	2	19	21	9.5
	2184	24	22	46	52.2
	2186	17	16	33	51.5
	2191	..	35	35	0.0
	2192	18	24	42	42.9
	2903	..	51	51	0.0
25	..	250	718	968	25.8

OFFSPRING LESS THAN 20 FLIES

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
7	..	22	88	110	20.0

TOTAL

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
32	..	272	806	1078	25.2

Thus, it can be considered as established that if the male descends from the pure normal line, the progeny will always be normal whatever may be the genetical structure of the female. On the contrary, males, directly

TABLE 9

SERIES	NUMBER OF NORMAL CULTURES	NUMBER OF "FEMALE" CULTURES	NUMBER OF CULTURES WITH OFFSPRING LESS THAN 20 FLIES
♂ from No. 1828 × ♀ from normal line	10	10	7
♂ from No. 1851 × ♀ from normal line	12	13	7
Total	22	23	14

descending from line 3, give a "female" offspring even when mated with pure normal females. Hence the inevitable conclusion, that in the present case the character of the numerical sex-relations is entirely determined by the genetic structure of the father. Whether the offspring will be normal or "female" depends only on the male taken for mating; the female does not play any part here. This was confirmed afterwards by matings of numerous males to each of several different females; the results obtained showed once more that the sex-ratio of the offspring is determined by the structure of the father only.

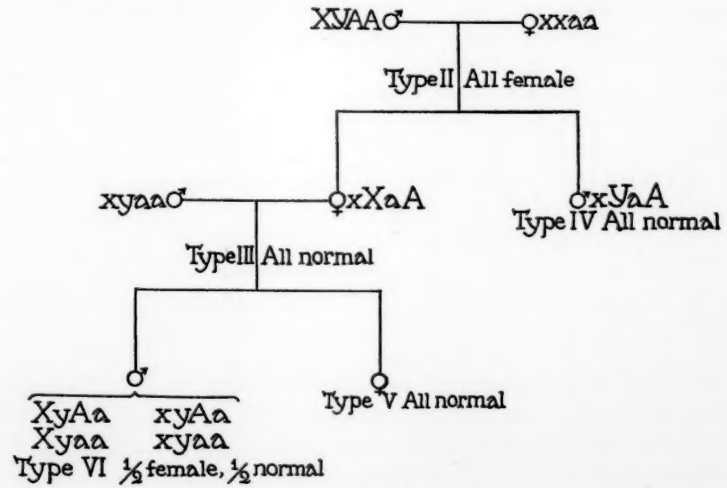

Figure 4

To explain the inheritance of the observed phenomenon by a violent alteration in the chromosomal apparatus, like non-disjunction, attachment of chromosomes, translocation or polyploidy, appears to be utterly impossible. Neither can plasmatic heredity explain the results of the crosses described. If we turn to the normal chromosomal mechanism, three hypotheses are possible. The factor, determining the abnormality studied, can be transmitted either by the Y or by the X-chromosome, or lastly by one of the autosomes. Figure 4 represents the inheritance of the sex chromosomes and the autosomes proceeding from line 3 (that is, those chromosomes capable of carrying the factor for the abnormality) throughout all the crosses described. For the sake of clearness, only one pair of autosomes is figured in the drawing. The chromosomes from line 3 are indicated by capitals.

It is easy to show that the Y-chromosome is not concerned in the transmission of this factor. The males that carry the Y-chromosome that comes directly out of line 3 nevertheless give a perfectly normal progeny (in type IV). On the other hand, males with a Y-chromosome originating from the pure normal line are capable of giving "female" cultures (type VI). This gives sufficient evidence that the Y-chromosome, whatever may be its origin, plays no part in the hereditary transmission of the gene calling forth the abnormality.

Examining the inheritance of the autosomes from line 3 we see that during the crosses described they could never attain a homozygous state. Consequently, if the factor which determines the appearance of the "female" ratios is transmitted by means of an autosome, it must be capable of acting in a heterozygous state; only thus could the appearance of abnormal sex relations in the crosses of type VI be explained. All the males from No. 1644 gave a "female" progeny; therefore they all carried the gene determining the abnormality. Even if we suppose them to have been only heterozygous for this gene, nevertheless at least half of their sons ought to have inherited it, in case it were really localized in one of the autosomes. However, their sons show in their offspring exclusively normal sex-ratios (in type IV). Therefore the supposition that the gene which causes the appearance of "female" culture lies in an autosome, must be rejected.

There remains the possibility of its being localized in the X-chromosome. For a verification of this, the males obtained from the cultures of type III must be examined. Half of these males carry the X-chromosome received from line 3; likewise, just the same quantity of males from type III have given "female" offspring. Were it possible to prove that the "female"

culture were obtained exactly from those males, which carried the X from line 3, this would prove the correctness of the supposition that the gene determining the abnormality is transmitted by the X-chromosome.

The remaining males from the cultures of type III bear the X-chromosome proceeding from the normal line. These males, according to their genotypical structure, must be subdivided into two groups. The males of one group do not in any wise differ from the males of the normal line: all their chromosomes are perfectly normal. It is evident that this group could not yield "female" cultures. The males of the other group have only the autosomes originating from line 3. As was already proved, the autosomes do not contain the gene producing the abnormality; hence these males likewise cannot give an offspring with the "female" sex-ratio.

Thus, only such males can produce a "female" progeny, as derive the X-chromosome from line 3 of *D. obscura*, and this includes all of them, because they quantitatively exactly correspond to the "female" cultures of type VI: half the cultures of type VI gave a "female" offspring, and half of the males from type III carry an X-chromosome from line 3. In this way, the supposition that the gene which determines the capacity of males to produce a "female" progeny is really localized in the X-chromosome, can be considered as established.

ACTION OF THE GENE PRODUCING THE ABNORMALITY

Analysis by hybridization has led to the conclusion that the factor which calls forth the appearance of "female" cultures is transmitted by the X-chromosome as an ordinary sex-linked gene. Turning to the action of this gene we see that, first of all, the males carrying it produce a progeny with a numerically irregular relation of the sexes. On the contrary, the females which contain it, whether in a heterozygous or a homozygous state, nevertheless remain perfectly normal. This can be seen, for example, in the crosses of type III for the heterozygous females, and in the cross No. 1644 (and the like) for homozygous ones. In this way, the hereditary factor which causes the appearance of "female" cultures is manifested exclusively in the males; in other terms, it is not only sex-linked, but likewise sex-limited.

In order to penetrate somewhat deeper into the mechanism of its action, all the known cases of sex-ratio abnormalities are to be kept in view. The diversity of these deviations can be reduced to a comparatively small number of causes, which may be classified as follows:

I. Causes that influence the normal mechanism of distribution:

1. Parthenogenesis.
2. Unequal participation of different kinds of gametes in fertilization.

II. Causes exercising no influence on the normal mechanism of distribution:

1. Destruction of the zygotes of one sex through the injury of the gametes, which participate in fertilization.
2. Destruction of the zygotes of one sex, owing to their containing lethal factors.
3. Different resistance of the sexes to external influences.
4. A complete transformation of sex, as the extreme degree of intersexuality.

In order to decide to what group the phenomenon observed in *D. obscura* belongs, all the possibilities ought to be examined.

It is easy to establish at once, that the causes, enumerated for the first and the last categories (parthenogenesis and complete sex transformation) are totally inadequate to explain this abnormality. Parthenogenesis is entirely impossible, there being here a transmission of the X-chromosome from the father to its offspring (for instance, in the crossings of type II). The supposition that the observed abnormality is caused by a complete transformation of sex must also be rejected. Not once did I find in my cultures any individual bearing intersexual characters, although all the flies underwent a careful examination; neither did the anatomical dissection of numerous flies from the "female" cultures present any abnormalities. Further, a male of Drosophila, transformed into a female, must give two kinds of gametes—those with a Y and those with an X-chromosome. The combination with similar gametes of a normal male would present four types of zygotes: XX, 2XY and YY (the last perishes). Consequently, in the progeny of such transformed males a sex-ratio of 1 female to 2 males ought to be found—a thing that was never observed during the present work. Lastly, in some cases (for instance in the crosses of type II) the missing males are such as ought to be perfectly normal in regard to their genotypical structure; therefore, their transformation is of course highly improbable.

Passing on to other possibilities, it is necessary first of all to answer the question: Does one of the sexes perish in the process of development, or are both kinds of zygotes produced in different quantites from the very beginning? Happily, in Drosophila the problem can be solved. As is shown by the genetic and physiological experiments of other investigators, the female of Drosophila lays all the fertilized eggs, whether they

be capable of development or not. By counting the number of eggs laid and then that of the flies developed out of them, one can determine the percent of the embryos that have perished and even the stage when their death took place. Such researches have been more than once carried out with *D. melanogaster* and the methods have been sufficiently well elaborated.

Table 10 gives results of analogous experiments undertaken to answer

TABLE 10

SERIES	NUMBER	EGGS	LARVAE	FLIES	EGGS DIED (percent)	LARVAE AND PUPAE DIED (percent)	PERCENT ♂'♂
Normal line	1780	108	106	74	1.9	30.2	51.4
	1783	84	80	58	4.8	27.5	46.6
	1785	127	127	89	0.0	29.9	44.9
	1936	105	101	69	3.8	31.7	47.8
	Total	424	414	290	2.4	30.0	47.6
Line 3	1940	125	123	85	1.6	30.9	5.9
	1943	135	130	81	3.7	37.7	2.5
	1944	85	85	55	0.0	35.3	3.6
	1951	98	98	77	0.0	21.4	5.9
	1959	67	64	49	4.5	23.4	6.1
	Total	510	500	347	2.0	30.1	4.6

this question in *D. obscura*. It was necessary firstly to determine the percent of perishing embryos in the cultures of the pure normal line; the number obtained were to serve as a control. The results showed that nearly all the eggs began to develop, and only 2 percent of them perished. On the contrary, the percent of larvae that succumbed was rather large; this may be attributed to the unsatisfactory nature of the diet. The experiments were carried on in a thermostat with a constant temperature of 25°C. The methods used were the same as described in the recent work of J. CHI LI (1927). A second series of experiments, carried out under the same conditions, was organized with flies homozygous for the gene which causes the appearance of "female" cultures. The results obtained here turned out to be extremely like the data given by the control; the percent of eggs and larvae that perished was almost exactly the same as in the normal line.

Thus, direct experiments, made in order to solve the question whether there exists in the "female" cultures of *D. obscura* a differential removal of one of the sexes in the course of development, give a clearly negative answer. Therefore it must be concluded that there is here a real alteration in the mechanism regulating the normal sex-destribution, that is, an alteration of the mechanism of sex-determination.

CONCLUSION

Analysis of the irregular sex-distribution in *D. obscura* shows that this phenomenon is due to the presence of a sex-linked gene. The action of this gene is analogous to that of a gametic lethal; the greater part of the spermatozoa determining the development of males do not participate in fertilization, consequently they are lost for the offspring. To say with greater precision on the strength of the results obtained, what constitutes the action of this gene, seems at present impossible, but nevertheless some suppositions on the subject can be made. Only two possibilities have to be reckoned with here: either the "female" progeny is determined by an unequal formation of spermatozoa with the X and Y-chromosomes during spermatogenesis; or else, even if the quantity of both kinds of spermatozoa formed be equal, the preponderance of females in the offspring can be explained by the inability of the sperms with the Y-chromosomes to compete with the sperms carrying the X. The last supposition seems more probable, as in this way the appearance of a certain number of males in the "female" cultures is easier to explain, but it is as yet impossible to decide finally to which of the two described types the observed phenomenon belongs. Anyhow, it can be considered as certain that the spermatozoa with the Y-chromosome participate in the fertilization process in but an insignificant number, while the spermatozoa carrying the X-chromosome (and therefore the gene that calls forth the abnormality) fecundate unhindered the great majority of eggs.

It is worth while to note that this gene was first obtained from nature. Out of 19 wild females which were caught two were heterozygous for it, thus giving us the right to suppose that it infects in a comparatively high degree the local *D. obscura* population. The gene is sex-linked, therefore it ought to respond to the influence of natural selection with particular directness, as no inbreeding is required for its manifestation. Hence the conclusion can be drawn that since it exists in the natural population it is probably useful, or at least harmless for the evolution of the given species. If this supposition be correct, the extension of this gene among the wild population must be expected even without the action of positive

selection. Usually a sex-linked gene is transmitted by the father to a half of the descendants only, whereas here all or nearly all the flies receive it with the X-chromosome of the father; this favors its extension.

It is interesting to note that an apparently quite similar sex-ratio abnormality was found by STURTEVANT in *Drosophila affinis* (MORGAN, BRIDGES and STURTEVANT, 1925). He gives the following description of this case: "The data obtained indicate that a great deficiency of sons was obtained from certain males, regardless of the source of the females to which such males were mated. The few males obtained from such matings were, usually at least, normal in behavior; but some of the sons of the females from such anomalous cultures again gave very few sons." Unfortunately this interesting stock was lost before a more detailed analysis of it could be undertaken, so that it is impossible to determine whether the causes of both the abnormalities are the same. However, it seems very probable that the mechanism of both cases must be identical at least in regard to its essential features.

SUMMARY

1. The sex-ratio in the normal lines of *Drosophila obscura* is very near to the theoretical 1:1.

2. Out of 19 females caught in nature, two were heterozygous for a gene which causes strong deviations in the normal sex distribution.

3. The researches made have shown that this gene is localized in the X-chromosome and is transmitted like an ordinary sex-linked gene.

4. This gene is absolutely sex-limited, as it is not manifested either in heterozygous or homozygous females.

5. The males bearing this gene give in their progeny about 96 percent of females and only about 4 percent of males.

6. This gene has no influence on the development of the zygotes already formed, but acts directly upon the mechanism of sex-distribution. It provokes a sharp preponderance of females by almost totally removing the spermatozoa with the Y-chromosome from the fertilization process, acting thus like a gametic lethal (in the genetic sense of this term).

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AN EIGHT-FACTOR CROSS IN THE GUINEA PIG*[1]

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THE STOCKS

An eight-factor cross in the guinea pig

The purpose of the present paper is to present data on the assortment, relative to each other and to sex, of eight pairs of Mendelian factors of the guinea pig. A stock, recessive in six color factors and in two factors which affect hair direction, was built up from various sources. This stock consisted of smooth furred, pink eyed, pale brown-yellow-white tricolors. A stock of self colored golden agoutis, smooth furred except for reversal of hair direction on the hind toes, was built up to supply the 8 dominant allelomorphs. The following are the series of factors involved.

S, s	s	piebald (white areas) (incomplete dominance
E, e^p, (e) e^p		tortoise shell (red areas), e self red
A, a	a	black in place of agouti
C, c^k, (c^d, c^r, c^a)	c^k	slight dilution of black, marked dilution of red
	c^d	marked dilution of both black and red
	c^r	slight dilution of black, white in place of red, red eyes
	c^a	white in place of both black and red, pink eyes (albino)

* The GALTON AND MENDEL MEMORIAL FUND pays part of the cost of the accompanying tables.

[1] A large portion of the data for this paper was obtained in experiments carried on by the author in the Bureau of Animal Industry, UNITED STATES DEPARTMENT OF AGRICULTURE.

B, *b*	*b*	brown in place of black, brown eyes, red unaffected
P, *p*	*p*	pale sepia in place of black, pink eyes, red unaffected
R, *r*	*R*	rough fur in place of smooth fur, at least on hind toes
M, *m*	*m*	extension of roughness to back and head (incomplete dominance of *M*)

DIFFICULTIES IN CLASSIFICATION

As might be expected, there were certain difficulties of classification among the 192 varieties expected in the backcross generation (*M*, *m* produce no visible effect except in the presence of *R*).

The piebald pattern (due primarily to *ss*) is subject to the action of modifiers which may cause the average percentage of white to vary from at least 10 to 90 percent. The average in the multiple recessive stock was 59 percent for males and 67 percent for females. There is also much variation which is not genetic and yet not due to environmental factors common to litter mates. Genetic piebalds (*ss*) occasionally show no white. It is not unlikely that homozygous self (*SS*) could be produced with a little white by selection for the appropriate minor factors. These cases were certainly so rare in the stocks used that there would have been no serious difficulty in classification into self and piebald were it not that dominance is incomplete. The heterozygotes are often completely self colored but rather more frequently they show a little white on nose or feet. Occasionally they range as high as 25 percent white in the coat, at least in females, with no more residual heredity for white than expected from the stocks used here. The distribution of percentages of white in the multiple recessive stock, in the F_1 matings of 3 homozygous dominant animals and in the backcross generation, are given in table 1. There is clearly some overlapping of the ranges of *Ss* and *ss* but separation of the males into "near self" and "piebald" at 7.5 percent white and of the females at 22.5 percent gives approximately equal numbers of the two classes and can involve no error that would seriously affect the recombination percentages.

The factor e^p (tortoise shell) was given preference over *e* (self red) in making up the multiple recessive stock, in order to avoid interference with the agouti series. Factor *e* was, however, carried in one of the foundation stocks, that used for introduction of *b*, and segregated out in a few backcross matings ($Ee \times e^pe$), in place of e^p. The distinction between the dominants (Ee^p) and the recessives (e^pe^p) from the typical backcross matings was ordinarily easy to make. There are rare cases (as noted by IBSEN 1919) in which animals which are undoubtedly e^pe^p show no red or yellow spotting in the coat, and more frequent cases in which there are

TABLE 1

The amount of white in the coat in (A) the multiple recessive stock (ss), (B) among F_1's from cross of multiple recessives with 3 multiple dominants proved to be homozygous (SS) by occurrence of no piebalds among 16, 24, and 26 young respectively, and (C) in the backcross generation excluding red-eyed dilutes.

MATING	SEX	AMOUNT OF WHITE IN COAT (parts in 20)																					
		0	x	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Multiple recessive stock (A) (*ss*×*ss*)	♂	2	5	6	6	10	9	6	6	3	10	10	8	11	19	12	14	15	10	16	10	18	17	223
1924, 1925	♀	2	4	1	4	6	5	5	6	6	4	7	9	15	13	16	20	14	12	16	21	25	20	231
3 F_1 Matings (*SS*×*ss*) (B)	♂	16	15	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	31
	♀	14	19	1	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	35
Backcross (*Ss*×*ss*) (C) (*c*^{c}*c*^{c} omitted)	♂	33	70	9	8	8	7	11	6	5	2	5	3	6	4	7	5	2	5	6	3	4	2	211
	♀	10	65	5	8	7	9	12	10	4	12	3	9	3	5	16	4	7	10	8	6	11	2	226

only a few hairs of these colors. The close approach to equality of recorded Ee^p and e^pe^p among dark-eyed young (115:113) indicates that there was little or no error among these but the deficiency of tortoise shells among the pink eyed ($134Ee^p$:$98e^pe^p$), although not certainly significant, may indicate some tendency to overlook yellow hairs in these animals in which black and brown are reduced to colors which do not contrast sharply with yellow. The crossover figures involving E are accordingly reported separately for dark-eyed and pink-eyed young. Another possible source of error in the separation of Ee^p from e^pe^p and also of Ss from ss came in the few cases in which factor c^r segregated out as the representative of the albino series. This factor reduces the red spotting of tortoise shells to white. Fortunately the pattern is typically so different from piebald that classification is usually possible. Among the 21 animals of formula c^rc^r, 2 were pink-eyed whites, resembling albinos, but undoubtedly of formula eec^rc^rpp. These, of course, could not be classified with respect to piebald. Five had no white spotting of any sort and could thus only be called $SsEe^p$, 3 had a trace of white only in regions expected in heterozygous piebalds and were also called $SsEe^p$, 4 had white brindling characteristics of tortoise shells, with little or no piebald white, and were called Sse^pe^p, 4 had a large amount of white in typical piebald patterns only and were called $ssEe^p$, while 3 showed both typical piebald white and white brindling and were called sse^pe^p.

There was never any doubt about the classification into agouti (Aa) and non-agouti (aa), except in 12 animals which showed no dark pigment in the coat (ee, or e^pe^p with so much white that there happened to be no dark pigmented spot).

In the albino series, factor c^k was given preference over c^r and c^a as the recessive representative. Albinism (c^a) would have interfered with the expression of all of the other color factors and c^r raises the difficulty in the separation of piebald and tortoise shell noted above. Factor c^d, which dilutes black more obviously than does c^k, would have been preferable, but these factors were not distinguished until the somewhat long drawn out process of putting the recessive stock together had been nearly completed. It turned out then that c^d had been wholly lost from this stock which thus came to be largely c^kc^k but carried c^r sufficiently frequently to enable c^rc^r to appear in the back cross generation in the 21 animals referred to above (from matings $Cc^r \times c^kc^r$). There was no difficulty in distinguishing Cc^k from c^kc^k in cases in which there was red (Cc^k) or yellow (c^kc^k) in the fur, whether due to the agouti pattern (Aa) or to tortoise shell (e^pe^p). In animals of constitution Ee^paa, the grade of black or brown averages

somewhat lighter with $c^k c^k$ than with Cc^k but as previously shown (WRIGHT 1925, 1927) there is so much overlapping that classification is unsatisfactory. It has seemed best to omit all animals of this constitution in considering the relations of the C series.

There was no difficulty in distinguishing *Bb* and *bb* in animals which were not pink-eyed as both eye color and the difference between black and brown in the dark parts of the fur provide reliable criteria. Eye color fails in the pink-eyed varieties and the difference between pale sepia and pale brown is not always clear cut. It has seemed best to present the data for dark eyed (*Pp*) and pink eyed (*pp*) separately, although it is believed that there was no substantial amount of error in the latter.

There was never any difficulty in distinguishing *Pp* with black, brown or red eyes, from *pp* with pink eyes, albinism having been entirely absent from the recessive stock. A brown-pale brown-red-white quadricolor, mosaic in *P* and *p*, appeared in the backcross generation (WRIGHT and EATON 1926). This animal transmitted *P* in a mating with a pink-eyed animal and is here classified as *Pp*.

There was also no difficulty in distinguishing rough furred (*Rr*) from smooth (*rr*) (except in two animals of which only the heads were not eaten when found).

The rough furred fell easily into two approximately equal groups, full roughs, *Rrmm*, and partial roughs *RrMm*. The former have at least two pairs of dorsal rosettes, and rosettes about and between the eyes, while in the latter there is typically only one dorsal pair or even merely a dorsal crest and no head rosettes. A few doubtful, intermediate cases were assigned by drawing a somewhat arbitrary line. This factor *M* was derived from a different source from the modifier of full rough described in an earlier paper (symbol *S*, WRIGHT 1916). Nevertheless its behavior as followed in the production of the multiple dominant stock was exactly the same. Inasmuch as a similar modifier has been found homozygous in 3 wild species of cavy as well as in various domestic stocks of guinea pig there need be little hesitation in considering it as always the same. Smooths of formula *rrMM* are to be considered as "type" from which the full rough *RRmm* differs by 2 factors. In the production of the multiple recessive stock, all of the final stock (smooth) was derived by segregation from full rough parents (*Rrmm*×*Rrmm*) in order to insure the absence of factor *M*.

ABSENCE OF SEX LINKAGE

The first crosses between the multiple dominant and recessive stocks were made before the former had been rendered completely homozygous.

There was in consequence segregation of one or more factors in most cases. In a typical case of a mating between a male dominant and a female recessive the young consisted of 11 partial rough golden agouti (4 ♂, 7 ♀) and 9 partial rough cream agouti (5 ♂, 4 ♀), indicating a mating of type *SSEEAACc^{a}BBPPRRMM*×*sse^{p}e^{p}aac^{k}c^{k}bbpprrmm*. The production of males dominant in all 8 factors in this and other similar matings proves that all 8 of the factors are transmissible from father to son and nence not sex linked, if the male is heterozygous for sex as in cats and man and as indicated by cytologic evidence for a number of other mammals. The cytologic situation is not certain in the case of the guinea pig. An X-Y pair is reported in the primary spermatocyte by STEVENS (1911) and by HARMON and ROOT (1926) but had not been identified in work reported by PAINTER (1926). However, it is clear from reciprocal crosses (female dominant by male recessive) that none of the 8 factors show sex linkage of the type in which the female is heterozygous for sex. A typical mating of this sort gave 8 partial rough agoutis (2 ♂, 6 ♀) and 4 partial rough agouti tortoise shells (all males) indicating segregation of *E* and *e^p* in the mother. Transmission of all 8 factors from mother to daughter was found in many other cases besides the 6 from this cross.

THE MONOHYBRID RATIOS

Fourteen F_1 males and 17 F_1 females, all partial rough agoutis with a little white in the fur in most cases, were tested by backcrossing to the recessive stock. The results for each pair of factors separately are given, by matings, in table 2. The males A, B and C and females a to i came from the cross of male dominant with female recessive, while the others came from the reciprocal cross.

There are two cases (matings F and T) in which it is clear from the results that the F_1 parent was not a heterozygous dominant with respect to piebald, but a recessive (*ss*). One of these (T) had 15 percent white in his coat and came from an F_1 mating which was proved to be *Ss*×*ss* by the production of well defined piebalds. The amount of white made it more probable than not that he would turn out to be a recessive in this respect, although another male (H) with 15 percent (as well as the male of G with 10 percent) turned out to be heterozygous. The rest of the males had only 5 percent or less white in the coat. The breeding record of mating F makes it certain nevertheless that this male (no white in coat!) also was genetically a piebald. The progeny of these matings are used in studying the recombination percentages except those involving *S*.

TABLE 2

Segregations with respect to each pair of allelomorphs separately from each F_1 male and F_1 female (some mated 2 or 3 times). Male of YZ was mated with Rr females and hence with expectation of 3R-:1rr instead of 1:1. Males of F and T proved to be ss. Females of u, v, and w proved to be AABBPP.

	NUMBER	♂	♀	*S*	*s*	*E*	$e^p e$	*A*	*a*	*C*	c^{kr}	*B*	*b*	*P*	*p*	*R*	*r*	*M*	*m*
A, G	30	10	20	14	16	18	12	13	17	4	14	20	10	9	21	12	18	5	7
B	9	6	3	6	3	3	6	5	4	3	4	4	5	4	5	3	6	0	3
F	13	9	4	(0	13)	6	7	7	5	7	4	7	6	6	7	8	5	3	5
H	12	7	5	4	6	3	9	2	4	6	4	5	4	6	6	6	6	1	5
I	28	13	14	16	11	14	13	11	17	11	9	17	11	14	14	14	14	7	7
J, Q, R	40	23	17	24	16	21	19	14	25	14	10	21	18	23	17	21	19	7	14
K	14	5	9	8	6	7	7	6	8	3	7	8	6	4	10	7	7	5	2
L, M	29	16	13	14	15	18	11	20	9	17	7	15	14	20	9	16	13	5	11
N	3	0	3	2	1	2	1	2	1	2	0	1	2	2	1	1	2	0	1
O	7	2	5	1	6	2	5	3	3	2	4	3	3	4	3	6	1	5	1
S, V	34	14	20	18	15	21	12	12	22	12	9	19	14	20	14	12	22	9	3
T	14	6	8	(0	14)	7	7	7	6	6	7	7	6	4	10	11	3	8	3
W, X	17	4	13	9	8	10	7	6	11	4	5	7	10	8	9	9	8	5	4
Y, Z	19	9	10	10	9	7	12	10	8	8	8	9	9	8	11	(12	7)	6	6
Total (1:1)	269	124	144	126	112	139	128	118	140	99	92	143	118	132	137	126	124	66	72
Dev. ± SE		10.0±8.2		7.0±7.7		5.5±8.2		11.0±8.0		3.5±6.9		12.5±8.1		2.5±8.2		1.0±7.9		3.0±5.9	
Prob.		.22		.36		.50		.17		.61		.12		.76		.90		.61	
(0:1) & (3:1) unclassified		1		(0 4	27)	2		11		78		8				(12	7)		

a	34	17	17	18	16	16	18	17	17	17	11	18	16	18	16	17	17	8	9
b	6	4	2	2	4	3	3	3	3	2	2	1	5	5	1	4	2	2	2
c	24	16	8	11	13	16	8	16	8	10	9	13	11	9	15	12	12	8	4
d	2	1	1	1	1	1	1	2	0	0	2	1	1	2	0	1	1	1	0
e	3	1	2	0	3	1	2	3	0	2	1	1	2	0	3	0	3	0	0
f	3	2	1	2	1	3	0	2	1	1	1	1	2	2	1	2	1	1	1
h	4	1	3	0	4	2	2	2	2	0	3	2	2	2	2	3	1	1	2
i	7	6	1	3	4	6	1	2	5	2	1	2	5	3	4	4	3	3	1
j	5	3	2	3	2	1	4	3	2	1	3	2	3	3	2	3	2	2	1
k	17	8	8	8	9	13	4	10	7	6	6	13	4	10	7	8	9	3	5
l, r	15	5	10	8	7	10	5	9	6	6	4	7	8	4	11	7	8	6	1
m	16	7	8	6	9	8	8	10	6	10	2	7	9	8	8	4	11	4	0
n, s	26	11	14	15	11	14	11	16	9	11	9	13	12	16	10	18	7	6	12
o	21	8	13	10	11	9	12	7	14	5	9	8	13	9	12	8	13	1	7
p	3	3	0	1	2	2	1	1	2	0	2	2	1	2	1	3	0	1	2
t	8	2	6	5	3	5	3	3	5	3	2	2	6	3	5	4	4	2	2
u, v, w	27	14	13	12	15	10	17	(26	0)	15	12	(27	0)	(27	0)	9	18	5	4
Total (1:1)	221	109	109	105	115	120	100	106	87	91	79	93	100	96	98	107	112	54	53
Dev. ± SE		0.0±7.4		5.0±7.4		10.0±7.4		9.5±6.9		6.0±6.5		3.5±6.9		1.0±7.0		2.5±7.4		0.5±5.2	
Prob.		1.00		.50		.18		.17		.36		.61		.88		.74		.92	
(1:0) unclassified		3		1		1		(26	0) 2	51		(27	0) 1	(27	0)	2			
(1:1) ♂ + ♀ tests	490	233	253	231	227	259	228	224	227	190	171	236	218	228	235	323	236	120	125
Dev. SE		10.0±11.0		2.0±10.7		15.5±11.0		1.5±10.6		9.5±9.5		9.0±10.7		3.5±10.8		1.5±10.8		2.5±7.8	
Prob.		.36		.85		.16		.89		.32		.40		.75		.89		.75	

TABLE 3

Progeny from backcross test of F_1 males. The matings are indicated by letters as in table 2 and the litter by a subscript. Matings F, T, Y and Z are put in parentheses. F and T come from F_1 males which proved to be genetically piebald (ss) and the male of Y and Z was mated with heterozygous rough females. The symbols have the following meanings: B—Black; pBr—pale Brown; W—White; (Br)—Brown eye; Br—Brown; R—Red; Ag—Agouti; (P)—Pink eye; pS—pale Sepia; Y—Yellow (or cream); (B)—Black eye; Sm—Smooth; RfA—Full Rough; RfC—Partial Rough. Such a combination as BrYAg-Y(Br) means brown yellow agouti with yellow spotting and (brown eye).

	NEAR SELF				PIEBALD							
	Full Rough		Partial Rough		Smooth		Full Rough		Partial Rough		Smooth	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
BRAg (B)		L_6		V_3	I_7	$J_3K_8L_3L_{12}$	M_2	Q_3				
BYAg (B)		I_9*	R_3	I_{10}		I_2*	(F_6)			V_3	$L_6(Y_1)$	A_8V_3
BrRAg (Br)	J_1		L_{12}						(Z_2)	H_2L_4	V_4	
BrYAg (Br)		A_2K_4			L_{10}						L_6	
B (B)	V_9	A_{10}	J_3	R_7S_1* V_9	Q_2X_2		(F_2)	X_3	$A_{10}M_2$	A_9V_8	$(F_1)I_6J_4L_9$	I_1*Q_3V_8
Br (Br)			L_1	J_4	$B_1K_7L_2$ R_7V_2	N_1Q_3	J_3	I_4		O_2	R_8V_3	K_7W_2
BRAg-R (B)		A_4S_1		O_1		N_1	L_2	R_8			L_7	
BYAg-Y (B)		Z_4			B_3I_4	H_3I_3*		(Z_1)		$A_7(T_3)$	A_7 I_6	O_1
BrRAg-R (Br)	$B_3(Y_2)$					$L_{11}X_1$					R_3	
BrYAg-Y (Br)					G_1J_4	B_2R_4	R_2					L_1W_1
B-R (B)	I_3R_3		X_3	$L_9W_5(Y_2)$	Q_2			(F_2)		(Y_3)		
B-Y (B)						W_5			V_1	(T_2)		
Br-R (Br)	M_1	I_5			$I_6V_5V_6$	$V_7(Z_3)$	H_3	(F_3)	I_6	$(T_3)_4$		V_4V_8
Br-Y (Br)	J_3		V_6	J_3					O_2			$L_7(T_2)$

pSRAg (P) pSYAg (P) pBrRAg (P) pBrYAg (P)		L_6 R_4 (Y_1)		(Y_4)	A_1A_6	K_1 V_6 L_3	(T_4) W_2 L_4 (F_4)	L_7 I_1 N_1 $B_3(T_1)V_8$	(T_3) G_1 (T_4)	(T_2) O_1 (F_8)X_3	(F_8) V_6	(T_4) I_9V_2 A_4I_{10}
pS (P)	H_4	R_6		A_3	$S_1S_1V_6(Z_2)$	V_2V_6		$A_1A_6G_1$ L_6W_3		(T_2)	R_3Q_1	Q_1
pBr (P)	H_2I_9 X_1	A_2	$I_7(Z_1)$	W_4	$A_3A_7A_9I_4$	K_2V_3	$R_2(Y_1)$		J_2	K_2X_2	B_3	$A_3I_{10}W_4$
pSRAg-R (P) pSYAg-Y (P) pBrRAg-R (P) pBrYAg-Y (P)	M_1 J_3	 L_4	I_7	 I_7	$A_6K_8(Z_4)$ R_6	K_6 $A_1(Y_2)$ W_2X_2	B_2L_6 (Y_4)		(F_1)R_1 (F_4)	V_4	(F_1) Q_3 V_7	A_6
pS-R (P) pS-Y (P) pBr-R (P) pBr-Y (P)	Q_2	R_5	I_4	I_5	$B_1I_2V_5$	A_6R_1 B_3R_6	I_7	K_4R_7*	O_2 K_3 T_1 K_3	K_5 (Z_4)	H_4 (F_3)(Y_1) $L_4(T_2)$	A_5I_1 A_9 A_4A_5

* White (due to c^rc^r) in place of yellow (due to c^kc^{kr})

Miscellaneous

♀	R-W	(B)	Sm	se-CBPr-	(H_3)	♀	R-W	(P)	Sm	Se-C-pr-	(Q_3)
♂	R-W	(B)	RfA	se-CBPRm	(H_4)	♀	Y-W	(P)	RfA	se-c^k-pRm	(Q_2)
♀	R-W	(Br)	RfA	se-CbPRm	(H_2)	♀	Y-W	(P)	Sm	se-c-^{k}p (r)	(Y_3)
♀	R-W	(P)	Sm	Se-C-pr-	(H_2)	—	pS-	(P)	RfC	--a-BpRM	(I_2)
♂	W	(P)	Sm	-e-c^r-pr-	(H_2)	♀	pSWAg	(P)	Sm	--Ac^r-pr-	(S_1)
♂	W	(P)	Sm	-e-c^r-pr-	(H_4)	♂	Y-W	(P)	RfA	(s) e^p-c^k-pRm	(T_3)
						♀	Y-W	(Br)	Sm	(s) e^p-c^kbPr-	(F_4)

TABLE 4

Progeny from backcross test of F_1 females. The tested females are indicated by letters as in table 2 and the litters by a subscript. The record of female u (including v and w) homozygous in A, B and P is given separately below. Color symbols as in table 3.

	NEAR SELF						PIEBALD					
	Full	Rough	Partial	Rough	Smooth		Full	Rough	Partial	Partial	Rough	Smooth
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
BRAg (B)			f_1			c_6	n_6		a_4			k_3
BYAg (B)	n_1	a_8		n_3	$k_6{}^*o_7$	d_1			r_4			h_2t_3
BrRAg (Br)		o_8	C_8						a_6c_3	n_1		b_1k_6
BrYAg (Br)	p_1				f_1	a_9			m_4	a_{10}		k_3n_8
B (B)	k_5				k_2	l_1	s_2	s_3			c_4	n_2s_2
Br (Br)	a_8	0_1	i_4i_4		c_2m_4	o_4s_4			b_2			m_3o_8
			n_1									
BRAg-R (B)								c_5			$a_{10}t_2$	a_6m_2
BYAg-Y (B)			$b_1j_3{}^*$					c_4s_2	a_2			
BrRAg-R (Br)	b_2				m_1	m_6	a_9			j_3	m_{37}	
BrYAg-Y (Br)	n_2				c_2o_1	l_1n_6	o_5	$k_5{}^*$	d_1		j_3	
B-R (B)	k_6		i_1		k_3	$a_{11}m_1r_1$						a_{11}
B-Y (B)	a_3						a_6	o_3	$p_1{}^*$	c_4		
Br-R (Br)	n_5				a_3a_5			t_3				
Br-Y (Br)		a_1			a_2				h_1			b_2 o_7
pSRAg (P)	a_7	s_1	r_3	a_2	c_5	k_1m_1	k_2				$c_5i_1m_3$	c_2
pSYAg (P)	c_2		k_4		a_5					r_2	c_3	
pBrRAg (P)	n_3		r_1			a_3t_1		r_3		m_6		
pBrYAg (P)		n_3			c_6	c_1					e_1k_6	$a_1c_2i_3$

pS (P) pBr (P)	$c_7 j_2$*		c_1 t_2*	a_7 $a_{11} o_2$ t_4	$a_3 l_1$	a_3 t_4*	p_1 $b_2 i_2$	h_2*k_3 f_1	$c_4 r_2$	k_4* m_6	$a_1 m_4 o_6$ $o_1 i_1$	$o_3 r_4$ r_1
pSRAg-R (P) pSYAg-Y (P) pBrRAg-R (P) pBrYAg-Y (P)	n_6	n_3 o_4		n_8 r_3	$m_6 o_8$	l_1		0_3 h_1	s_1 c_6	c_6 a_4	a_6	e_1 $k_5 r_1$ $a_7 e_1$
pS-R (P) pS-Y (P) pBr-R (P) pBr-Y (P)		a_{10} t_2*			c_7 n_3 o_6	o_7 j_1*		a_4 o_8	c_8	s_4 m_5	$a_4 o_8$	o_2

* White (due to $c^r c^r$) in place of yellow (due to $c^k c^{kr}$).

BRAg (B) BYAg (B) BRAg-R (B) BYAg-Y (B)		u_8		u_4 $u_3 u_8$	$u_1 u_4$ $u_1 u_5$ $u_3 u_3$	u_6 u_9	u_7 u_6	u_7	u_2 u_8		u_2 u_{10} $u_6 u_7$	u_6 $u_{10} u_{10}$ $u_2 u_7 u_9$

no BRAg spot present.

Miscellaneous

♂ R-W (Br) Sm se-CbPr- (s_2)
– pSRAg (P) -S-AC-p-- (s_3)
– pSRAg -R-W (P) - -e^pACB^p-- (m_2)
– B (B) RfC SEc-BPRM (k_6)

One F_1 female (mating r) produced no non-agoutis (*aa*) no browns (*aa*) and no pink-eyed (*pp*) among 27 young and was clearly *SsEe*^{p}*AACc*^{k} *BBPPR*^{r}*Mm*. There was no doubt about her identity because of a certain peculiarity in her rosette pattern recorded at birth and there was nothing in the records to indicate that her dam (a multiple dominant) had ever mated with any other male than her reputed sire (a multiple recessive). Whatever may be the explanation, it has seemed safe to include her progeny in the recombination data except where A, B and P are concerned.

One of the F_1 males was mated with two females which were full roughs (heterozygous) although recessive in the seven other respects (matings Y and Z). The progeny are used in estimating all crossover percentages except those involving *R*.

Examination of the totals in table 2 shows that the monohybrid ratios are all reasonably close to 1:1 although as noted above it is not unlikely that a few genetic tortoise shells are classed as self. The chance of obtaining a more divergent system of values for the 9 cases (including sex) is .85 by the χ^2 method (9 degrees of freedom). It must be remembered, however, that in the case of *S*,*s* and *M*,*m*, the overlapping classes were separated somewhat arbitrarily.

THE DIHYBRID RATIOS

The results of the backcross generation from the F_1 males are given in detail in table 3, the mating and litter of each animal being indicated by the entries. Table 4 deals similarly with the progeny of the F_1 females. All of the data are included either in the body of the table or below. The cases which cannot be used for every crossover test are indicated as described in the titles.

The combinations suitable for determining possible linkage relations between each pair of factors are abstracted in tables 5 and 6 together with the calculations of the crossover percentages and the chances of exceeding each by random sampling. Inspection of these reveals not one, either in the male or female tests, which can be said to differ significantly from 50 percent. The most probable cases of linkage among the male tests are those of *AM* and *BR* with indicated crossover percentages of 43.4 and 45.3 percent respectively. The chance of exceeding by random sampling are .08 and .14 in the two cases. The reality of these cases is not confirmed by the female test. The strongest indication among the latter is that of *C* and *B*, 45.8 percent with a probability of being exceeded by chance of about 1 in 3 and no confirmed by the close approach to 50 percent (49.2 percent) among the males. It will be seen that the deviations

TABLE 5

The combination of each two sets of allelomorphs in backcross tests of F_1 males. The two original combinations are indicated in the column headings by XY and xy (double dominant and double recessive respectively) while the cross combinations are indicated by Xy and xY following in the order of the factors in the first column. The backcross ratio (1:1:1:1) is disturbed in the case of EC and AC by omission of Ea in which the separation of C and c[k] was not satisfactory and in the case of RM by omission of smooths in which M and m produce no visible effect at all. The last column gives the probability of obtaining at least as great a deviation from 50% in either direction by random sampling. The value of χ^2 from the numbers of crossovers and linkages is 20.0 indicating a probability of .86 of obtaining at least as great deviations by random sampling (28 degrees of freedom).

	XY	Xy	xY	xy	TOTAL NO.	CROSSOVERS		PROBABILITY
						No.	Percent	
SE	64	62	62	50	238	124	52.1	.52
SA	56	68	47	60	231	115	49.8	.95
SC	49	38	37	40	164	75	45.7	.27
SB	70	54	58	52	234	112	47.9	.51
SP	68	58	54	58	238	112	47.1	.36
SR	51	65	55	48	219	120	54.8	.16
SM	26	31	28	33	118	59	50.0	1.00
EA	62	77	55	62	256	132	51.6	.62
EC	32	30	67	61	190	97	51.1	.77
EB	73	66	69	52	260	135	51.9	.54
EP	66	73	66	62	267	139	52.1	.50
ER	70	62	55	61	248	117	47.2	.37
EM	35	40	30	32	137	70	51.1	.80
AC	57	61	37	25	180	98	54.4	.23
AB	68	49	73	67	257	122	47.5	.42
AP	59	59	69	71	258	128	49.6	.90
AR	54	54	68	64	240	122	50.8	.80
AM	25	36	41	32	134	77	57.5	.08
CB	55	42	48	38	183	90	49.2	.82
CP	53	46	42	50	191	88	46.1	.28
CR	52	39	37	47	175	76	43.4	.08
CM	26	32	20	21	99	52	52.5	.61
BP	72	71	60	58	261	131	50.2	.95
BR	74	60	50	59	243	110	45.3	.14
BM	40	39	26	31	136	65	47.8	.61
PR	62	62	64	62	250	126	50.4	.90
PM	35	33	31	39	138	64	46.4	.39
RM	60	66	..	..	126	66	52.4	.59

TABLE 6

The combination of each two sets of allelomorphs in the backcross tests of F_1 females. Headings as in table 5. The value of χ^2 from the numbers of crossovers and linkages is 13.2 indicating a probability of .992 of obtaining at least as great deviations by random sampling (28 degrees of freedom).

	XY	Xy	xY	xy	TOTAL NUMBER	CROSSOVERS		PROBABILITY
						Number	Percent	
SE	60	44	60	55	219	104	47.5	.46
SA	49	44	56	43	192	100	52.1	.56
SC	45	35	45	44	169	80	47.3	.49
SB	42	50	50	50	192	100	52.1	.56
SP	48	45	48	52	193	93	48.2	.61
SR	51	53	56	59	219	109	49.8	.94
SM	25	26	29	27	107	55	51.4	.77
EA	59	51	46	36	192	97	50.5	.89
EC	37	32	53	47	169	85	50.3	.94
EB	54	56	39	44	193	95	49.2	.83
EP	49	61	47	36	193	108	56.0	.10
ER	60	60	47	52	219	107	48.9	.74
EM	33	27	21	26	107	48	44.9	.29
AC	56	50	19	17	142	69	48.6	.74
AB	52	53	41	46	192	94	48.9	.77
AP	53	53	42	45	193	95	49.2	.83
AR	52	52	46	41	191	98	51.3	.72
AM	27	25	22	24	98	47	48.0	.69
CB	40	35	30	37	142	65	45.8	.31
CP	35	41	40	27	143	81	56.6	.11
CR	39	50	40	49	168	90	53.6	.35
CM	21	18	18	22	79	36	45.6	.43
BP	45	48	51	49	193	99	51.3	.72
BR	45	47	53	47	192	100	52.1	.56
BM	20	25	29	24	98	54	55.1	.31
PR	49	47	49	47	192	96	50.0	1.00
PM	24	25	25	24	98	50	51.0	.84
RM	54	53	..	..	107	53	49.5	.92

from 50 percent are even smaller on the whole than might be expected by chance.

As already noted, the classification of factors *E* and *B* was easy in dark-eyed animals but was more difficult and accompanied occasionally by a feeling of uncertainty in the pink-eyed ones. It seems desirable therefore to examine the crossover percentages of these groups separately. The

TABLE 7

The crossover percentages from tests of F_1 males in the cases involving E and B, divided according to the presence of Pp or pp. In the former case, separation of Ee^p from E^pe^p and Bb from bb was easy and wholly reliable. In the latter cases there was more difficulty and occasionally uncertainty.

	BLACK OR BROWN EYED YOUNG (*Pp*)				PINK EYED YOUNG (*pp*)			
	Total Number	Crossovers		Prob.	Total Number	Crossovers		Probability
		Number	Percent			Number	Percent	
SE	122	65	53.3	.47	116	59	50.9	.85
SB	122	62	50.8	.86	112	50	44.6	.25
EA	128	67	52.3	.60	128	65	50.8	.86
EC	95	52	54.7	.36	95	45	47.4	.61
EB	132	58	43.9	.16	128	77	60.2	.02
EP	132	66	50.0	1.00	135	73	54.1	.34
ER	124	62	50.0	1.00	124	55	44.4	.21
EM	68	30	44.1	.33	69	40	58.0	.19
AB	128	55	43.0	.11	129	67	51.9	.66
CB	95	50	52.6	.61	88	40	45.5	.39
BP	132	60	45.5	.30	129	71	55.0	.25
BR	124	55	44.4	.21	119	55	46.2	.41
BM	68	32	47.1	.63	68	33	48.5	.81

TABLE 8

The crossover percentages from tests of F_1 females in cases involving E and B, divided according to the presence of Pp or pp. In the former case separation of Ee^p from e^pe^p and Bb from bb was easy and wholly reliable. In the latter case there was more difficulty and occasionally uncertainty.

	BLACK OR BROWN EYED YOUNG (*Pp*)				PINK EYED YOUNG(*pp*)			
	Total Number	Crossovers		Probability	Total Number	Crossovers		Probability
		Number	Percent			Number	Percent	
SE	123	57	46.3	.42	96	47	49.0	.84
SB	96	51	53.1	.54	96	49	51.0	.84
EA	95	46	48.4	.76	97	51	52.6	.61
EC	102	50	49.0	.84	67	35	52.2	.71
EB	96	48	50.0	1.00	97	47	48.5	.76
EP	96	47	49.0	.84	97	61	62.9	.01
ER	123	59	48.0	.65	96	48	50.0	1.00
EM	58	24	41.4	.19	49	24	49.0	.89
AB	95	50	52.6	.61	97	44	45.4	.36
CB	75	37	49.3	.91	67	28	41.8	.18
BP	96	51	53.1	.54	97	48	49.5	.92
BR	96	46	47.9	.68	96	54	56.3	.22
BM	49	26	53.1	.67	49	28	57.1	.32

necessary data are given in tables 7 and 8. The crossover percentages are somewhat more variable, as expected with the smaller numbers, but none differ significantly from the expectation under random sampling either in the dark-eyed or pink-eyed, except perhaps the figure of 62.9 percent for E and P in pink-eyed young in the female test and that of 60.2 percent

TABLE 9

The crossover percentages from tests of F_1 males and F_1 females combined. The value of χ^2 from the numbers of crossovers and linkages is 12.25 indicating a probability of .995 of obtaining at least as great a series of deviations from equality by random sampling (28 degrees of freedom).

	TOTAL NUMBERS	CROSSOVERS		PROBABILITY
		Number	Percent	
SE	457	228	49.9	.96
SA	423	215	50.8	.73
SC	333	155	46.5	.21
SB	426	212	49.8	.92
SP	431	205	47.6	.31
SR	438	229	52.3	.34
SM	225	114	50.7	.84
EA	448	229	51.1	.64
EC	359	182	50.7	.79
EB	453	230	50.8	.74
EP	460	247	53.7	.11
ER	467	224	48.0	.38
EM	244	118	48.4	.61
AC	322	167	51.9	.50
AB	449	216	48.1	.42
AP	451	223	49.4	.81
AR	431	220	51.0	.66
AM	232	124	53.4	.29
CB	325	155	47.7	.41
CP	334	169	50.6	.83
CR	343	166	48.4	.55
CM	178	88	49.4	.88
BP	454	230	50.7	.78
BR	435	210	48.3	.47
BM	234	119	50.9	.79
PR	442	222	50.2	.92
PM	236	114	48.3	.60
RM	233	119	51.1	.74

for *E* and *B* in the pink-eyed young in the male test. The former figure may readily be explained by the failure to recognize a small yellow patch in a few pink-eyed tortoise shells.

As only slight differences have been found in the crossover percentages of males and females in mammals (rats, mice and rabbits) it is probably legitimate to combine the male and female tests. The results are given in table 9. Here again there is no case which differs significantly from 50 percent, the extremes being 46.5 percent and 53.7 percent. The probability of obtaining a system of at least as great deviations (χ^2 test) by random sampling is the embarrassingly high figure of .995. The grand average recombination percentage for the 28 comparisons is 49.9 percent. It appears that for the present these 8 factors and sex must be considered as isolated representatives of 9 different linkage systems. There remain, of course, the possibility that some factors may be found showing appreciable linkage with two of the above and thus binding them into the same system.

THE OCTOHYBRID RATIO

Before leaving the data there are two other aspects from which it will be desirable to examine the randomness of assortment. First consider the combinations with respect to all 8 factors instead of merely by 2's.

The data are condensed in table 10 by disregarding the sex of the F_1 parent and of the offspring. Matings F, T, Y, Z and v are omitted because of the imperfections discussed above and mating D because of the production of 6 self reds in which classification by the agouti series could not be made. The few other imperfect individual records are also omitted. The 398 young left include 143 of the 168 distinguishable varieties expected in the backcross generation. Among the 96 classes in which expectation is 1/256 (or 1.56 in 398) there is variation from 0 to 4 individuals. Among the 56 classes with expectation 2/256 (or 3.11 in 398) there is variation from 0 to 8. The 8 classes with expectation of 4/256 (6.22 in 398) vary from 4 to 11 individuals. As these variations may appear greater than probable, it is worth while testing them against the distribution expected under random sampling which is approximately that of the Poisson series for mean values of 1.56, 3.11 and 6.22 respectively. Table 11 shows the comparison between observation and expectation on this basis. The χ^2 test, combining adjacent groups so as to leave none smaller than 4, indicates a probability of getting at least as great a system of divergences by random sampling of .33. Clearly there are no important departures from a random distribution.

TABLE 10

Segregation in backcross generation, combining tests of F_1 males and F_1 females, ignoring sex and omitting records of two males (F, T) found to be ss, of one male (H) found to be Ee instead of Ee^p and producing 6 ee young unclassifiable with respect to A and a, and one female (of u, v, w) proved to be AABBPP, and one male (YZ) which was mated with females heterozygous for Rough (Rr). All of the young from the other matings are included except 7 which were unclassifiable in one or more respects.

CHARACTERS		GENES	1 NEAR SELF			PIEBALD		
			Rf AB	Rf CD	Smooth	Rf AB	Rf CD	Smooth
			SRm	SRM	Sr-	SRm	sRM	sr-
BRAg	(B)	*EACBP*	1	2	6	3	1	1
BYAg	(B)	*C^kB*	3	3	4	0	2	5
BrRAg	(Br)	*Cb*	2	2	0	0	4	3
BrYAg	(Br)	*c^kb*	3	0	3	0	2	3
B	(B)	*Ea−BP*	3	5	4	3	4	9
Br	(Br)	*−b*	2	5	11	2	2	6
BrRAg−R	(B)	*e^pACBP*	2	1	1	3	0	5
BYAg−Y	(B)	*c^kB*	1	2	3	2	2	3
BrRAg−R	(Br)	*Cb*	2	0	4	1	1	2
BrYAg−Y	(Br)	*c^kb*	1	0	8	3	1	3
B−R	(B)	*e^paCBP*	3	4	5	0	0	1
B−Y	(B)	*c^kB*	1	0	1	2	3	0
Br−R	(Br)	*Cb*	3	0	6	1	2	2
Br−Y	(Br)	*c^kb*	2	2	1	0	2	3
pSRAg	(P)	*EACBp*	3	2	4	2	0	4
pSYAg	(P)	*c^kB*	1	1	4	2	3	1
pBrRAg	(P)	*Cb*	2	1	3	3	2	2
pBrYAg	(P)	*c^kb*	1	0	2	2	0	8
pS	(P)	*Ea−Bp*	3	3	6	8	1	8
pBr	(P)	*−b*	3	6	9	4	6	7
pSRAg−R	(P)	*e^pACBp*	1	1	3	2	3	3
pSYAg−Y	(P)	*c^kB*	1	1	3	1	0	2
pBrRAg−R	(P)	*Cb*	2	2	1	0	2	3
pBrYAg−Y	(P)	*c^kb*	2	0	3	1	1	1
pS−R	(P)	*e^paCBp*	1	1	6	2	1	4
0S−Y	(P)	*c^kB*	1	1	3	2	2	2
pBr−R	(P)	*Cb*	1	0	2	0	1	0
pBr−Y	(P)	*c^kb*	1	0	1	1	3	3

COMPARISON OF LITTERMATES

It is next desirable to see whether the data show any tendency toward resemblance of littermates in factorial constitution. In particular it is

TABLE 11

Distribution of class frequencies in Table 10 under each mean Mendelian expectation (1/156, 2/256, or 4/256) compared with random distribution as calculated from Poisson series with means of 1.56, 3.11 and 6.22 respectively. χ^2 for 12 degrees of freedom is 11.44 indicating a probability of 0.33 of obtaining at least as great deviations from theory by random sampling.

NUMBER IN CLASS	CLASSES WITH MEAN MENDELIAN EXPECTATION OF								
	1/256 1.56 in 398			2/256 3.11 in 398			4/256 6.22 in 398		
	O	C	O−C	O	C	O−C	O	C	O−C
0	22	20.3	+1.7	3	2.9		0	0.0	
1	29	31.5	−2.5	10	8.9	+1.2	0	0.1	
2	29	24.5	+4.5	10	13.8	−3.8	0	0.3	
3	14	12.7	+1.3	20	14.3	+5.7	0	0.6	
4	2	4.9	−5.0	8	11.1	−3.1	1	1.0	
5	0	1.5		5	6.9	−1.9	0	1.2	
6	0	0.4		5	3.6	+1.9	2	1.3	−1.5
7	0	0.2		0	1.6		1	1.1	+1.5
8	0			3	0.6		1	0.9	
9	0			0	0.2		2	0.6	
10	0			0	0.1		0	0.4	
11	0			0			1	0.5	
Total	96	96.0		64	64.0		8	8.0	
Individuals	137			201			60		

important to determine whether the number of littermates which resemble each other in all factors, including sex, is so great as to indicate the frequent occurrence of uniovular twins. Table 12 shows the number of agreements between littermates classified as ♂♂, ♂♀ and ♀♀ with respect to the S, E, A, B, P and R series. The M series is omitted because the possibility of classification of two littermates in this respect requires that both be rough furred and the C series is omitted for similar reasons. Matings F, T, Y, Z, and U are, of course, omitted. The symmetry of the results about 3 agreements, 3 disagreements in each case indicate that agreement is largely by chance. This has been tested by combining all into one table treating sex as a 7th factor and comparing the distribution with the expansion of $(\frac{1}{2}A+\frac{1}{2}D)^7$ where A and D are agreements and disagreements respectively. The close approach of the observed data to the theoretical distribution is obvious and is confirmed by a probability from χ^2 of .90. There is thus no tendency for littermates to resemble each other more than animals picked at random.

TABLE 12

Distribution of agreements and differences between littermates in sex and the six series S, E, A, B, P and R. Comparison is made with expectation from random sampling based on the expansion of $(\frac{1}{2}A+\frac{1}{2}D)$.[7] *Matings F, T, Y, Z and u, v, w, are omitted because of inequality of chances of agreement and difference in one or more of the above respects. The probability of obtaining at least as great deviations from theory by random sampling is 0.90 from* $\chi^2=1.65$ *for 5 degrees of freedom.*

AGREE	DIFFER	♂♂	♂♀	♀♀	TOTAL	*Calculated*	DIFFERENCE
0	7	..	3	..	3	*3.1*	−0.1
1	6	2	17	0	19	*21.3*	−3.2
2	5	9	49	11	69	*64.0*	+5.0
3	4	25	50	25	100	*106.6*	−6.6
4	3	32	45	28	105	*106.6*	−1.6
5	2	25	16	25	66	*64.0*	+2.0
6	1	6	3	17	26	*21.3*	+4.7
7	0	2	..	0	2	*3.1*	−1.1
		101	183	106	390	*390.0*	0.0

There were, however, two cases in which there was agreement in sex and in the 6 factors. There was indeed an 8th agreement in each case. Two male littermates, both brown with a trace of white were both partial roughs and thus both ♂*SsEe^{p}aabbPpRrMm* with uncertainty only in the albino series. Two other male littermates were both smooth pink-eyed pale sepias, one with no white, the other with a trace. While differing distinctly in grade of sepia, both were so pale as to be probably c^kc^r (both ♂*SsEe^{p}aac^{k}c^{r}BBpprr*). These two cases may have been uniovular twins but as 3 cases of agreement in all of the respects dealt with in table 12 are expected by chance, little confidence can be placed in this. It is certain that such twins are so rare as to be of no importance in disturbing the randomness of Mendelian results.

DISCUSSION

The evidence for random assortment of *S*, *E*, *A*, *C*, *B*, *P*, *R*, *M* and sex, presented here is in harmony with the rather scanty data hitherto published. CASTLE (1916) found a close approach to random assortment in 108 F_2 animals from a cross of golden agouti (wild Cavia cutleri) (*EEAACCBB*) and albino guinea pig, *eeaac^{a}c^{a}bb*. There was also random assortment in 116 F_2's from a cross of *Cavia Cutleri* with brown-eyed creams, *eeaac^{d}c^{d}bb* as far as *E*, *A*, and *B* were concerned, classification by intensity (*C*, *c^d*) not having been attempted. The present writer (1916) found 32/75=43 percent recombination between *R* and *M*, 34/53=64

percent between *R* and *A* and 35/64 = 55 percent between *A* and *M*, and found recombination to occur between *R* and *E*, *B*, *C* and *P* and between *M* and *B*, *C*, *P* and in a later paper (1923) noted the completely random assortment *S* and *E* in 11 successive backcrosses to an inbred tricolor stock. IBSEN (1923) reported the following ratios of non-crossovers to crossovers:

	*N**	*X**		*N**	*X**		*N**	*X**
EA	5	10	AC	7	4	CP	65	67
EC	43	55	AB	9	4	CR	22	27
EB	19	9	AP	7	9	BP	10	18
EP	82	73	AR	9	5	BR	10	11
ER	29	31	CB	11	6	PR	47	28

*n = noncrossover. x = crossover.

IBSEN and GREGORY (1924) report independent assortment of *P* and a factor *Sm* (salmon eye) not belonging to any of the series discussed here and the present writer (1927) has found no linkage between another color factor, *F* and factors *C* and *P* among 242 F_2's, which data incidentally gave further evidence of the complete independence of factors *C* and *P* of each other.

The most interesting cases are perhaps this case of *C* and *P* and that of *C* and *B*. Factors with effects similar to those of *C* and *P* in the guinea pig have been found to be linked in both the mouse and rat with crossover percentages between 10 and 20 percent in both cases (CASTLE 1919). The albino series (*C*) is linked with the brown series (*B*) in the rabbit (41 percent crossover) (CASTLE 1924). The apparent absence of linkage in these cases in the guinea pig does not necessarily prove that the factors are not homologous in the different rodents as there may well have been radical chromosome reorganizations since their remote origin from common ancestry, a phenomenon indicated by probable differences in chromosome number.

The chromosome number in guinea pigs, however, seems to be far from certain. Estimates published in the past 20 years range from 8 for the haploid number (LAMS 1913) to 30 (PAINTER 1926). STEVENS (1911) found 28 and HARMON and ROOT (1926) found 19. The independence of the first 9 factors tested in the guinea pig (including sex) is difficult to reconcile, with a haploid count of 8 but is not at all surprising if one of the larger numbers proves correct.

SUMMARY

Production of two stocks of guinea pigs carrying 8 dominant and 8 recessive factors, respectively, made possible a simultaneous test for possible linkage relations between each two of the factor series designated by *S*, *E*, *A*, *C*, *B*, *P*, *R*, and *M*.

A backcross generation of 269 individuals from the (largely) octohybrid males showed completely random assortment and similarly with a backcross generation of 221 from (largely) octohybrid females. None of the factors showed sex linkage either of the XY type or the ZW type.

143 of the 168 visibly distinct combinations expected in the backcross generation were obtained among 398 young from completely octohybrid backcrosses. It is shown that the distribution of the combinations, considering all factors at once, is random.

There is no indication of a tendency for littermates to resemble each other more than expected by chance. Two possible cases of uniovular twins were found where three cases of resemblance in all non-interfering respects was expected by chance.

The independence of the first 9 factors tested is not surprising if the large number of chromosomes reported for the guinea pig recently is correct.

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A SEX DIFFERENCE IN CHROMOSOME LENGTHS IN THE MAMMALIA[1]

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During the last two years we have had occasion to accurately draw and enlarge human chromosomes. Both male and female, embryonic and adult, tissues have been studied. In surveying the first or longest chromosomes of the series we were early struck by the greater length of this chromosome in the male. Five independent series of measurements have furnished invariable confirmation of the earlier observations.

There are many pitfalls for the investigator who aims at accuracy and comparable results with the mensuration of chromosomes. Prophase chromosomes are invariably slightly longer than those assembled at the equatorial plate after dissolution of the nucleus; varying methods of fixation and dehydration shrink tissues unequally; and finally there are differences in the length and size of chromosomes in various cells especially in embryonic cells as compared with adult ones. It is rather interesting that different types of cells vary somewhat in chromosome length as they undoubtedly do in the size of the cell itself. A striking instance of this is found in the lutein cells in the corpora lutea of the ovary at the time of pregnancy. The cells are greatly enlarged and the chromosomes have a corresponding increase in size, the length and diameter being approximately two or more times those of the chromosomes of the surrounding cells. For all of these reasons we have sought to compare chromosome lengths only in the same phase of mitosis (late prophase) from the same type of tissue (mesenchyme) in embryos of approximately the same age, fixed and dehydrated by identical processes. Many human embryos of both sexes were explored for our work, but an immediate fixation (at the operating table) with resulting clarity of the chromosomes was secured in only two embryos of approximately the same age; a male of G. L. 19.5 to 20.5 mm and a female of G. L. 25 mm.

In both cases we selected ten nuclei of the mesenchyme situated near the ectoderm, displaying clearly all of the forty-eight chromosomes, which were then individually drawn at 3600 diameters. The original

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drawings were enlarged twice photographically. From these records measurements were made of the chromosome lengths, which were determined by measurement of the median axis in every case.

There is a clearly marked sex difference in the length of the first (or longest) pair of chromosomes. The mean length of the two longest chromosomes from the ten male nuclei exceeded by 8.22 mm the mean length of the longest female pair. The probable error of this difference is + .74 mm. The difference is thus over ten times the probable error of the difference. It indicates that the longest autosomes in this male embryo

TABLE 1

Individual measurements (×7200) of extreme autosomes (in mm) of ten nuclei from human embryos of each sex.

NUCLEUS	LONGEST PAIR				SHORTEST PAIR			
	Male		Female		Male		Female	
1.	37.6	37.5	23.7	23.5	7.5	8.3	9.3	9.7
2.	38.9	33.0	35.6	31.0	8.1	9.0	10.3	11.3
3.	39.6	33.5	28.0	25.2	6.5	8.0	10.0	10.6
4.	38.2	36.1	31.1	28.0	7.3	9.0	8.7	9.1
5.	38.5	36.1	34.2	30.2	9.1	9.5	8.7	9.7
6.	38.0	34.7	39.3	27.2	7.8	8.5	9.5	9.5
7.	41.0	40.7	28.7	28.5	10.0	10.0	10.3	10.7
8.	40.4	37.5	36.5	31.0	8.0	9.8	10.0	10.1
9.	45.6	37.7	30.7	29.0	8.1	8.7	9.0	9.0
10.	39.5	38.5	30.0	26.7	8.7	9.0	10.2	11.2
Average	39.7	36.5	31.8	28.0	8.1	9.0	9.6	10.1

TABLE 2

Human embryos (10 nuclei of each sex; measurements in mm).

AUTOSOMES CONSIDERED	MEAN	STANDARD DEVIATION
Longest pair ♂	38.13± .41	2.73± .29
Longest pair ♀	29.91± .62	4.08± .44
Difference ♂ − ♀	8.22± .74	−1.35± .51
Average of all 46 autosomes ♂	18.79± .24	7.46± .17
Average of all 46 autosomes ♀	17.23± .16	5.17± .11
Difference ♂ − ♀	1.56± .30	2.29± .21
Shortest pair ♂	8.55± .13	.90± .10
Shortest pair ♀	9.86± .11	.72± .08
Difference ♂ − ♀	−1.31± .17	.18± .12

were significantly longer than the longest autosomes in this female embryo. The data are given in table 9, and the constants computed from them, in table 2.

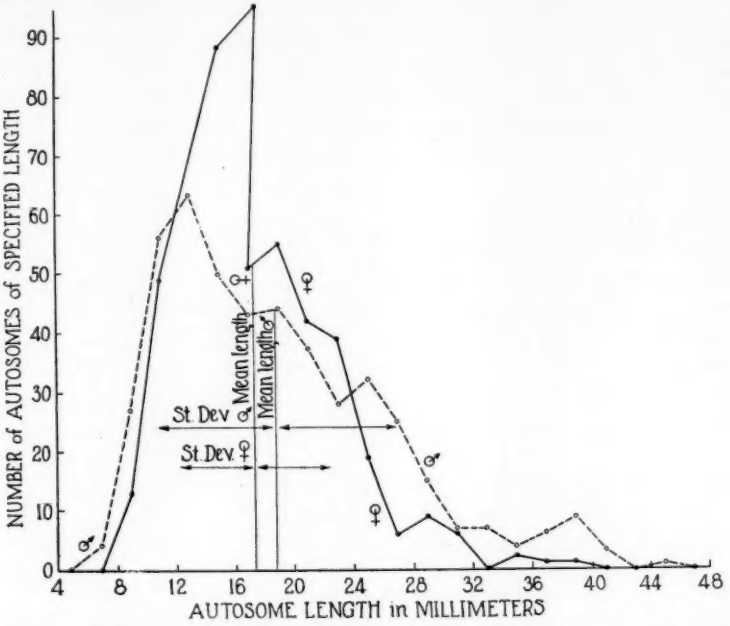

FIGURE 1.—Frequency distribution of autosome measurements in human embryo nuclei; all 46 autosomes in 10 nuclei of each sex.

It will be noted that the standard deviations are not significantly different in the two sexes when only one pair of autosomes is considered. When all twenty-three autosome pairs were studied (neglecting the XX and XY pairs) and the mean autosome lengths computed, the excess in length of male autosomes is seen to be much less than that shown in the case of the longest (first) pair. A difference of only 1.56 mm results. The probable error of the difference is .30 mm. Thus, though this difference is small it is also significant, being over five times its probable error. The frequency distributions of these measurements are given in table 3.

It is interesting to note, both from the frequency distributions and the computed standard deviations, that, when all twenty-three pairs of autosomes are considered, the male autosomes show a much greater variability than the female autosomes. The difference between the standard devia-

TABLE 3

Frequency distributions of autosome measurements

AUTOSOME LENGTH IN MM	ALL THE AUTOSOMES—10 NUCLEI				LONGEST PAIR ONLY			
	Human embryos		Rat embryos		100 nuclei from one individual rat embryo		10 nuclei from each of 10 rat embryos	
	Male	Female	Male	Female	Male	Female	Male	Female
4.0 to 5.9	..	..	2	..	..	..	..	..
6–	4	..	5	1	..	..	..	..
8–	27	13	13	8	..	..	..	..
10–	56	49	41	49	..	..	..	..
12–	63	79	77	71	..	..	..	..
14–	50	88	82	78	..	..	..	..
16–	43	51	82	75	..	..	..	..
18–	44	55	81	71	..	..	..	..
20–	37	42	48	51	..	..	..	..
22–	28	39	52	53	..	2	..	..
24–	32	19	26	44	..	10	..	1
26–	25	6	15	34	4	17	..	3
28–	15	9	13	18	3	28	2	20
30–	7	6	15	19	15	37	8	29
32–	7	..	6	12	22	41	7	56
34–	4	2	9	6	29	25	18	29
36–	6	1	9	3	43	22	32	30
38–	8	1	3	4	22	11	43	17
40–	3	..	2	..	19	4	24	7
42–	..	..	6	..	20	3	24	5
44–	1	..	4	1	9	..	17	2
46–	..	..	3	2	6	..	10	1
48–	..	..	..	..	7	..	3	..
50–	..	..	2	..	..	..	7	..
52–	..	..	2	..	..	..	1	..
54–	..	..	..	..	1	..	1	..
56–	..	..	1	..	..	..	..	..
58–	..	..	1	..	..	..	..	..
60–	..	..	..	..	..	..	3	..
Total	460	460	600	600	200	200	200	200

tions is $2.29 \pm .21$ mm so that the difference is about ten times the probable error of the difference. The same sex difference in variability is shown by the coefficients of variation, which are, respectively, 39.70 ± 1.02 mm and $30.01 \pm .72$ mm.

This greater variability is, in fact, so great that the sex difference in mean autosome length is actually reversed when the shortest pair of autosomes is considered. This fact is, of course, indicated by the much

smaller sex difference in mean autosome length which was noted when all twenty-three pairs of autosomes were averaged, as compared with the sex difference when the longest pair only was considered.

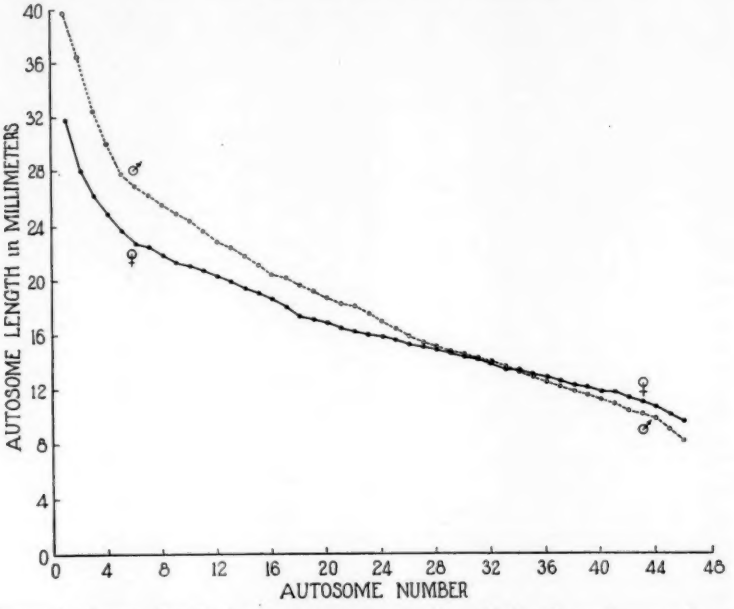

FIGURE 2.—Average lengths of successive autosomes in 10 nuclei of each sex—human embryo.

It thus becomes of interest to consider the smallest pair only. These data also are presented in table 1 and the constants in table 2. In this case the autosome lengths in the female *exceeded* those in the male by 1.31 $\pm$.17 mm. The difference thus indicates that in this male embryo the shortest autosomes were significantly shorter than the shortest autosomes of the female embryo.

Since the standard deviations are again not significantly different, the greater variability of the lengths of the autosomes of the male indicates a greater variation or range in length of the autosomes within a single nucleus, and not a greater variation between lengths of corresponding autosomes in different nuclei. Table 4, showing the averages and standard deviations of the twenty-three pairs in each of the ten nuclei of each sex, is presented to corroborate these points. The standard deviations for the individual nuclei of the male range from 6.64 to 8.06 mm with a

value for the total distribution of 7.46 ± .17 mm; those of the female range from 4.03 to 5.69 mm, with a value for the total distribution of 5.17 ± .11 mm. The difference in standard deviations is thus 2.29 ± .20 mm. The difference in variability is therefore highly significant. Figure 1, plotting the frequency distributions, means, and standard deviations of the autosome lengths and figure 2, plotting the average lengths of the successive autosomes, show these sex differences graphically.

TABLE 4

Average of all 46 autosomes in each individual nucleus (in mm) in ten nuclei of human embryos of each sex.

NUCLEUS	MALE		FEMALE	
	Mean	Standard deviation	Mean	Standard deviation
1.	18.80 ± .69	6.94 ± .49	15.83 ± .40	4.03 ± .28
2.	17.57 ± .70	7.02 ± .49	17.39 ± .54	5.46 ± .38
3.	17.48 ± .76	7.62 ± .54	16.65 ± .49	4.89 ± .34
4.	17.52 ± .66	6.64 ± .47	16.09 ± .51	5.17 ± .36
5.	19.83 ± .70	7.08 ± .50	18.17 ± .55	5.57 ± .39
6.	20.17 ± .73	7.32 ± .51	16.70 ± .55	5.55 ± .39
7.	17.61 ± .80	8.06 ± .57	17.17 ± .47	4.68 ± .33
8.	21.17 ± .80	8.04 ± .57	18.09 ± .57	5.69 ± .40
9.	18.74 ± .68	6.88 ± .48	18.57 ± .54	5.40 ± .38
10.	18.74 ± .79	7.94 ± .56	17.61 ± .42	4.23 ± .30
Average	18.79 ± .23	7.46 ± .17	17.23 ± .16	5.17 ± .11

We were interested in seeing whether this sex difference in chromosome lengths, so evident in embryonic tissue, is demonstrable in the tissue of adults. Unfortunately tissue growth in the male, save in the testis, (spermatogenesis), consists merely in the relatively slight normal reparative processes seen, for instance, in the epithelia of the skin and gut. We hence sought neoplastic growths in boys or men and finally secured a labial carcinoma in a man of 55 years, where mitotic division was rife. The use of this material is unfortunately open to the charge of "embryonic character" of neoplastic growth. In the case of the female, normal reparative processes in the endrometrium of early post menstruum furnished abundant instances and stages of dividing cells.

Measurements were made of chromosome lengths in ten nuclei of each sex. Here again the longest autosome pair of the male exceeded that of the female by ten times the probable error of the difference (table 6). When the lengths of the twenty-three pairs of autosomes were averaged,

TABLE 5

Individual measurements of extreme autosomes (in mm) in ten nuclei of human adults of each sex.

NUCLEUS	LONGEST PAIR				SHORTEST PAIR			
	Male		Female		Male		Female	
1.	40.4	37.1	27.2	26.3	7.0	10.0	6.7	9.3
2.	47.9	38.1	34.3	33.0	9.0	9.3	10.1	10.3
3.	37.2	37.0	30.1	25.8	10.5	11.0	9.1	9.3
4.	38.0	31.0	28.8	25.3	10.0	10.5	11.1	12.0
5.	39.0	34.5	28.9	28.3	13.1	14.8	10.0	10.3
6.	36.0	30.3	30.6	30.5	10.5	11.5	8.5	9.2
7.	50.0	44.8	31.6	31.5	9.2	10.2	11.0	11.4
8.	42.0	40.5	36.8	32.8	10.0	12.1	8.5	9.5
9.	37.3	33.5	24.0	22.7	11.4	12.0	7.5	7.5
10.	44.3	44.0	32.3	29.2	8.9	10.2	8.3	8.9
Average	41.2	37.1	30.5	28.5	10.0	11.2	9.3	9.8

TABLE 6

Human adult (10 nuclei of each sex, measurements in mm).

AUTOSOMES CONSIDERED	MEAN	STANDARD DEVIATION
Longest pair ♂	39.15 ± .76	5.07 ± .54
Longest pair ♀	29.49 ± .54	3.58 ± .38
Difference ♂ − ♀	9.66 ± .93	1.49 ± .66
Average of all 46 autosomes ♂	20.12 ± .22	7.15 ± .16
Average ot all 46 autosomes ♀	16.46 ± .16	5.24 ± .12
Difference ♂ − ♀	3.66 ± .27	1.91 ± .20
Shortest pair ♂	10.56 ± .25	1.63 ± .17
Shortest pair ♀	9.53 ± .18	1.21 ± .13
Difference ♂ − ♀	1.03 ± .31	.42 ± .21

the difference between male and female (3.66 mm), though smaller in the case with the longest pair only (9.66 mm), is nevertheless, also ten times its probable error. Even the shortest autosome pair in these adult autosomes was longer in the male than the female, though the sex difference here is by no means as great as with the longest pair. It will be remembered that in the embryonic mitoses the shortest male autosomes were shorter than the female ones. The mensurations of adult chromosomes showed, as did the embryonic, a much greater variability in male autosome lengths within a given nucleus as compared with female, whether measured by standard deviations or by the coefficients of variation. These measure-

ments thus furnish a confirmation of Darwin's belief in a greater variability in the male as a general law.

TABLE 7

Average of all 46 autosomes in each individual nucleus (in mm) in ten nuclei of human adults of each sex.

NUCLEUS	MALE		FEMALE	
	Mean	Standard deviation	Mean	Standard deviation
1.	18.61±.65	6.51±.46	16.39±.46	4.58±.32
2.	19.26±.79	7.97±.56	17.30±.65	6.54±.46
3.	19.87±.61	6.15±.43	15.61±.47	4.77±.34
4.	20.04±.69	6.92±.49	17.30±.43	4.29±.30
5.	20.52±.48	4.85±.34	17.52±.43	4.34±.31
6.	18.96±.55	5.49±.39	14.13±.46	4.60±.32
7.	21.39±.89	8.92±.63	18.09±.48	4.86±.34
8.	21.30±.74	7.47±.53	17.74±.58	5.87±.41
9.	20.96±.68	6.85±.48	14.30±.44	4.38±.31
10.	20.30±.86	8.65±.61	16.17±.58	5.85±.41
Average	20.12±.22	7.15±.16	16.46±.16	5.24±.12

Though we had taken all of the precautions possible to insure reliability in our conclusion of increased length of the longest male autosomes, the number of actually different animals studied was so small that we sought added evidence through the study of another mammalian form (the rat) and through a more rigorous control of all the factors influencing so delicate a task.

A pregnant rat was chloroformed on the twentieth day of gestation. The successive uterine enlargements were rapidly and deftly opened with fine tweezers and scissors, the embryos being instantly plunged under Bouin's fluid at 39°C. In each case the central body wall was cut widely open, enabling an immediate view of the gonads and diagnosis of sex to be made. We took care to secure identity in the fixation, dehydration, and all technical procedures involved in sectioning and staining the male and female litter mate embryos. Here again, in each sex, we delineated all the chromosomes in ten prophase mesenchyme nuclei located near the skin ectoderm and ideally fixed. As one of us has discovered (SWEZY, 1927), the hybrid rat of our colony possesses both sixty-two and forty-two chromosomes instead of the single count of forty-two which, in agreement with PAINTER (1927), we find the albino possesses. The rat chromosomes were all magnified 8400 diameters for measurement. The data are presented in table 8 and the constants in table 9.

TABLE 8

Individual measurements (×8400) of extreme autosomes (in mm) in ten nuclei from rat embryos of each sex.

NUCLEUS	LONGEST PAIR				SHORTEST PAIR			
	Male		Female		Male		Female	
1.	44.8	42.0	32.7	32.2	8.3	8.5	10.7	10.7
2.	46.7	41.1	44.6	39.6	8.0	8.2	11.0	11.1
3.	39.5	39.3	35.2	33.7	7.3	7.3	8.5	9.5
4.	57.8	45.8	46.5	37.8	6.5	10.7	8.2	10.0
5.	59.3	51.6	31.3	30.6	10.7	11.2	9.7	10.0
6.	52.1	46.5	32.8	32.5	10.7	10.8	7.5	8.8
7.	44.0	42.2	36.1	33.6	10.1	10.6	10.7	10.8
8.	42.3	36.9	33.1	34.1	4.5	7.6	10.6	10.6
9.	45.3	43.9	47.5	38.3	5.0	9.0	10.0	10.0
10.	53.2	42.0	36.0	35.8	6.0	9.4	8.7	10.2
Average	48.5	43.1	37.6	34.8	7.1	9.4	9.5	10.2

TABLE 9

Rat embryo (10 nuclei, measurements in mm).

AUTOSOMES CONSIDERED	MEAN	STANDARD DEVIATION
Longest pair ♂	45.81± .89	5.92± .63
Longest pair ♀	36.20± .72	4.80± .51
Difference ♂ − ♀	9.61±1.14	1.12± .81
Average of all 60 autosomes ♂	19.71± .22	8.16± .16
Average of all 60 autosomes ♀	19.61± .18	6.38± .12
Difference ♂ − ♀	.10± .28	1.78± .20
Shortest pair ♂	8.24± .45	2.98± .32
Shortest pair ♀	9.84± .18	1.22± .13
Difference ♂ − ♀	1 .60± .49	1.76± .34
	Rat Embryo (100 nuclei from one individual)	
Longest pair ♂	37.87± .24	5.03± .17
Longest pair ♀	32.39± .20	4.11± .14
Difference ♂ − ♀	5.48± .31	.92± .22
	Rat Embryo (10 nuclei from each of ten different individuals)	
Longest pair ♂	40.30± .26	5.45± .16
Longest pair ♀	34.02± .18	3.82± .13
Difference ♂ − ♀	6.28± .32	1.63± .21

Here again a clear difference exists in the case of males and females in the length of the first autosome pair, the difference between the means being 9.61 mm with a probable error of 1.14 mm. The difference is thus approximately nine times the probable error of the difference. The difference between the standard deviations is insignificant just as in the human nuclei when only one pair of autosomes is considered.

In the case of the rat, when the entire sixty or forty chromosomes were studied (omitting the XX and XY pairs) and a grand mean thus established, the superiority in length of the male autosomes practically completely disappears. The difference between the means is then merely .10 mm, with a probable error of .28. Thus, in the rat, as in man, the sex difference in autosome length is reversed in the smaller pairs of autosomes. The difference between the standard deviations is $1.78 \pm .20$ mm. The autosomes of the male rat, like those of man, thus show a greater variability than those of the female. The coefficients of variation are here $41.40 \pm .93$ mm and $32.53 \pm .69$ mm.

Computing the constants for the shortest pair only, the actual excess in length of the *female* autosomes is found to be $1.60 \pm .49$ mm. The difference in standard deviations is in this case significant, the first case in which a significant sex difference in variability had been found when only one pair of homologous autosomes in different nuclei is considered. The difference is $1.76 \pm .34$ mm.

To dissipate any lurking suspicion that we had consciously or unconsciously *selected* male prophase nuclei with longer autosomes than in the female, we decided to study a hundred nuclei from each sex, considering the two longest autosomes only. It does not seem to us remotely possible that biased choice could have been exercised by us in such an extensive record. The results of these measurements gave us a difference between the average length of the male and the female first autosome pair of 5.48 mm, with a probable error of only .31 mm. The difference between these means is highly significant, being approximately eighteen times its probable error. The frequency distributions of these measurements are given in table 3. The standard deviations are here again significantly different.

As a fifth test of this sex difference, it was thought desirable to secure corresponding measurements in a large number of different individuals. Therefore the longest pair of autosomes was measured in 10 nuclei from each of 10 different rat embryos of each sex. The averages for the different individuals are listed in table 10; the constants computed from the entire series, in table 9; and the frequency distributions of the individual measurements in table 3. This series of measurements again shows the

same sex difference, the longest autosomes of the male being significantly longer than the longest autosomes of the female. The difference in this case is $6.28 \pm .32$ mm, which is nearly twenty times the probable error of the difference. The difference is nearly ten times the probable error of the difference when the average for each embryo is treated as a single observation. (It will be noted that the individuals from which the 100

TABLE 10

Average lengths of longest pair of autosomes in ten different rat embryos of each sex.

EMBRYO NUMBER	AVERAGE OF 10 NUCLEI OF EACH EMBRYO			
	Male		Female	
1	39.8	35.4	39.4	34.9
2	50.1	44.5	40.0	35.1
3	39.6	37.4	35.8	32.5
4	41.7	38.1	35.0	32.1
5	39.3	36.7	33.1	31.2
6	43.1	38.4	35.0	30.9
7	46.0	42.6	34.0	31.3
8	39.8	35.2	35.0	32.8
9	41.9	38.2	32.9	30.9
10	41.7	36.2	35.8	32.8
Grand Average	42.3	38.3	35.6	32.5

TABLE 11

Rat Embryo (10 nuclei, measurements ($\times 8400$) in mm).

AUTOSOMES CONSIDERED	MEAN	STANDARD DEVIATION
Longest pair ♂	$45.81 \pm .89$	$5.92 \pm .63$
Longest pair ♀	$36.20 \pm .72$	$4.70 \pm .51$
Difference ♂ − ♀	9.61 ± 1.14	$1.12 \pm .81$
Average of all 60 autosomes ♂	$19.71 \pm .22$	$8.16 \pm .16$
Average of all 60 autosomes ♀	$19.61 \pm .18$	$6.38 \pm .12$
Difference ♂ − ♀	$.10 \pm .28$	$1.78 \pm .20$
Shortest pair ♂	$8.24 \pm .45$	$2.98 \pm .32$
Shortest pair ♀	$9.84 \pm .18$	$1.22 \pm .13$
Difference ♂ − ♀	$1.60 \pm .49$	$1.76 \pm .34$
	Rat Embryo (100 nuclei)	
Longest pair ♂	$37.87 \pm .24$	$5.03 \pm .17$
Longest pair ♀	$32.39 \pm .20$	$4.11 \pm .14$
Difference ♂ − ♀	$5.48 \pm .31$	$.92 \pm .22$

nuclei were measured happened to be near the lower end of the range of variation displayed by the 10 different embryos in this last series, while the individuals from which the first 10 nuclei were measured happened to be near the upper end of the range.)

Thus in five independent series of measurements, comprising the measurements of all the autosomes of ten nuclei of one individual of each sex in human embryo, human adults, and rat embryos, and the measurements of the longest pair of autosomes in one hundred nuclei from one rat embryo of each sex, and from ten nuclei from each of ten rat embryos of each sex, consistent and highly significant sex differences were found. The same sex difference in length of the longest autosomes is shown but not commented upon in part of the figures in PAINTER'S (1924) plate of the chromosomes of the monkey.

A study is being made of the reduction divisions in spermatogenesis to see whether this sex difference is due to a selective segregation of the chromosomes in the spermatids. This will be reported upon at a later date.

SUMMARY

1. In man and in the rat a significant and consistent sex difference occurs in the length of the autosomes of the somatic cells of both the embryo and adult.

2. The longest pair of autosomes in the male is longer than the longest pair in the female. This difference may be related to the greater body size generally found in the male.

3. Males also show a greater variation in the length of the autosomes within an individual nucleus. This difference could not be predicted from the greater body size of the male.

4. Thus, in addition to their possession of different chromosomes—the so-called sex determining chromosomes, the XX and XY pairs— male and females differ in the character (length) of the autosomes. This difference occurs in the early embryo and persists in the adult. It probably appears at the time of fertilization and can be looked upon as a "secondary" sex character inpressed from the beginning upon every cell in the body

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A CONSIDERATION OF VARIEGATION

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The segregation of albino or chlorophyll deficient seedlings in a pedigree propagated by self-fertilization is due either to a definite genetic factor or factors according to DEMEREC (1923) in maize, HALLQVIST (1926) in barley, etc., or to the abnormality of the plastids themselves according to CORRENS (1909) in Mirabilis, BAUR (1909) in Pelargonium, ANDERSON (1923) in maize, CLAUSEN (1927) in Viola, etc. In the former cases, the albino segregates occur in one-fourth, seven-sixteenths, one-sixteenth and so on, according to the number and behavior of the factors by which the deficiency of chlorophyll is manifested, while in the latter their segregating proportion is quite irregular, varying according to the degree of variegation on the parental plants from which the seeds were collected, or, in other words, the abnormal plastids conduct themselves independently of the genetic factors, their transmission therefore being non-Mendelian. Professor Sô (1921) studying the genetics of variegated barley made its peculiar behavior clear. His conclusions published in the Japanese language induced me to write this paper, in which I will give a critical discussion of the complicated heredity of some variegated plants in the light of Professor Sô's work and of analogous cases from observations made by myself.

I

Before entering into the main discussion, I will describe the results gained in my provisional studies on the variegated barley and rice. Once there came to my attention a case of the ordinary non-Mendelian variegation in barley. In 1923 I found a plant with coarse yellow stripes distributed over some leaves and an ear, and transplanted this to our experimental field to study its genetic nature. Owing to its poor growth and to transplantation at the flowering time, the seeds obtained were not numerous. From the green ears I raised 38 green seedlings, on which later no yellow stripes developed, and from the yellow part of a variegated ear 8 seedlings were obtained, all yellow, which died a few weeks after their germination.

Variegated seedlings are often found in rice, *Oryza sativa*, though they are rare in barley. In most cases the variegation occurs in coarse yellow stripes and is apt frequently to disappear in later growth. I collected the

seeds from a variegated rice plant and obtained 155 seedlings containing 48 yellows or 30.97 percent, the others being pure green, excepting one seedling which had a few yellow stripes on the lower leaf. The variegation on this specimen, however, completely disappeared in the later leaves and pure green ears only were produced. Althouh I did not carry out hybridization experiments with the green-and-yellow-variegated barley and rice, their non-Mendelain nature is certain from the abnormal segregation in their self-propagated progeny. The continuation of variegation of the ordinary non-Mendelian type by seeds is difficult in rice, and this is closely connected with its particularly coarse pattern of variegation. The cause of the variegated pattern, coarse or fine, of the non-Mendelian type was explained logically by WINGE (1919), who states in this connection: "Elementary mathematics will tell us that cells with a small number of green and white plastids will, by constant bisection in the course of a few cell generations, possibly even on the first division, give off both purely albinotic and self-colored green cells in the descendance, and only a very few of a mixed character, later none at all—whereas cells with but a slightly (not greatly) superior number of green and white plastids will only after a several cell generations, when the embryo has reached a considerable size, give off albinotic and self-colored green cells, and will never in the course of the ontogenesis lack cells with mixed contents" (p. 15). The different distribution of the abnormal plastids will give the corresponding difference in the proportion of variegateds in the offspring. Barley and rice stand at one extreme in regard to the small number of plastids contained in a cell, among the known non-Mendelian variegated plants. If this is the case the variegation should give a coarse pattern and the specimens should give a progeny containing green and yellow seedlings with a few or no variegateds, as actually recorded. On reading the subsequent sections we must bear in mind that the sorting out of the homogeneous plastids will occur very easily in barley and rice in the course of cell multiplication.

II

Among the specimens of barley cultivated in our College there is a variegated variety named "Okina" (old man). The foliage and ears of this variety are variegated with fine white stripes, and its varietal name came from its ears bearing some long white awns, reminding one of an old-man's white hair. The progeny of this variety contains some white seedlings, which die in a few weeks after germination when the food stored up in the endosperm is consumed. The proportion of albino

seedlings varies to some extent according to different samplings, though, on the whole, it is almost fixed. The seedlings, other than albinos, invariably grow up to be variegated. Observation on fully grown plants shows constancy in these respects. The variegated characteristic is segregated as a Mendelian recessive to the normal green. Sô (1921) studied thoroughly the genetic behavior of the variegation of this variety, and his main results, freely translated from the Japanese, are as follows:

1. The albinism is transmitted only maternally, the pollen having no immediate connection with it.
2. Variegation behaves as a recessive to green.
3. In F_2 and later generations, the segregation of variegation occurs in a mononhybrid ratio.
4. In crossing experiments, the pollen of pure white glumes is not different in its effect from that of green ones of the variegated ear.

On the basis of these experimental facts he developed his theory to account for the special behavior of the variegated barley in inheritance. The variegated character is transmitted as a recessive genetic factor, but it is not a pattern factor in a strict sense, because the white parts or stripes are due to the distribution of white plastids which have changed their quality permanently. The variegation is, therefore, produced by a factor which alters at times the essential quality of the plastids from normal green to colorless. The original difference between green and variegated barley lies in their allelomorphs for the stability of the plastid, and the variegation is due to the development of white stripes, owing to the distribution of the white plastids controlled no longer by genetic factors. To be more particular, let us designate the allelomorphs concerning the plastid mutability by *G* (dominant) and *G'* (recessive). Normal green barley is *GG*, and the variegated barley *G'G'*. The plastids of both are originally quite normal in their essential quality. Each of them is designated by *g*. It is assumed that in the presence of *G'G'* (double recessive) some of the green plastids mutate at times to white plastids (*w*). The plastids of self-green barley are all *g*, while those of the variegated barley consist of *g* and *w*. The former (*g*) may subsequently be altered in the presence of the factors *G'G'* to become white.

The plastid mutation, that is sometimes observed in various plants, is due in almost all cases to sporadic events and does not seem to be a habitual phenomenon. In the "Okina"-barley, however, the plastid mutation repeats itself so frequently in the course of the development of the plant body that not a single individual or even a leaf or an ear,

which is free from the variability or self-green, is produced. The variegated segregates appear in a recessive proportion from green hybrid plants. According to Sô's experiments, the white plastids are transmitted maternally. Under such circumstances, the reciprocal crossings of the normal and variegated varieties give partly different results in F_1, while practically the same in the later generations. Figure 1 is a diagrammatic

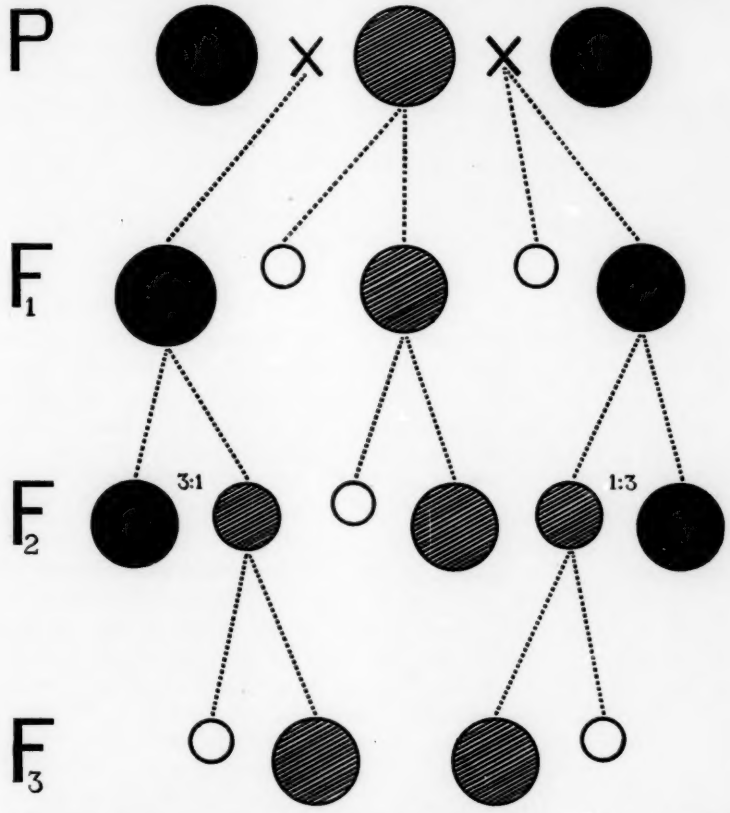

FIGURE 1.—Showing the heredity of variegated barley of the "special" type. The black circle indicates the green plants, the barred the variegated and the blank circle the albinotic. The size of the circles roughly represents the proportion of the respective segregates in a pedigree. The left group of F_1, F_2 and F_3 shows the results of cross-breeding of green (♀)×variegated (♂), those in the center the inbreeding of the variegated, and those at the right the cross-breeding of variegated (♀)×green (♂).

summary of inbreeding and cross-breeding of the "Okina"-barley, drawn from the results obtained by Sô. In barley the chance that white plastids and green ones are present in an egg-cell of a variegated plant may not be frequent, because the sorting out of homogeneous plastids will occur very easily in the foregoing cell-division, and yet the seedlings other than albinos are finely variegated, due to new plastid mutations occurring in their later embryonic stage. Sometimes the variegation of the seedlings is not clear, but successive observation make its existence manifest. The absence of pure white leaves and ears on the field-grown variegated barley, according to my opinion, may be regarded as due to the non-mutability of plastids in cells of the primitive ontogenic stage, including the rudiments of leaves and ears. As a result of this, there is no chance of obtaining pure white leaves or ears developed from white cells forming their rudiments. The absence of plastid mutability seems also to be the case in gametogenesis as well as in later somatic divisions. The plastid mutation occurs repeatedly during the development of the somatic tissues, excepting its very earlier and later times, and by the sorting out of the albinotic plastids the white stripes appear on the foliage. But owing to the non-mutability in the later somatogenesis the purity of cells for their plastids will be maintained. As a natural sequence we should obtain almost exclusively two kinds of gametes, the one containing all green plastids and the other all whites, by the variegated plants, in the same way as those of the ordinary non-Mendelain type. The data obtained by the crossing variegated (♀) × green (♂), which give rise to pure green and albino seedlings in F_1, comfirms this view. Practically, however, a few seedlings assuming a sectorial mosaic of white and variegated (or white and apparently green), though they rarely occur, are found among the progeny of variegated barley under my observation. According to my view, they must be regarded as developed from egg-cells containing green and white plastids together. If such seedlings are carefully cultivated we should obtain either white leaves or white ears, which are never found in the field-grown variegated barley in question, so far as our observation goes. Summing up, the plastid mutation period seems to begin at a late embryonic stage but ceases in later somatogenesis.

The variegation of the barley, therefore, is due to the distribution of a non-Mendelian albinotic character, but the causes of its repeated occurrence is due to a Mendelian factor. The genetics of this variegated barley is not comparable with the ordinary variegations studied in various plants, the case being "special" in its behavior. The variegation

in my yellow-striped barley was coarse, but that of "Okina"-barley is relatively fine; the difference is accounted for by the different genetic conditions by which the variegation appears.

III

Recently KONDÔ, TAKEDA and FUJOMOTO (1927)[1] have published a paper on the genetics of variegated rice from pedigree cultures of this plant. Their green-and-white-variegated races of rice are grouped under two classes in regard to hereditary type; in one the variegateds reappear in great numbers in the progeny, and in the other practically no variegateds are present but segregation produces green and white seedlings. The latter may be identified with that described in Section I. Among their experiments, A and B are treated principally as one and the same, therefore I will consider them together in the following discussion. In these experiments they obtained 64.5 percent of albino seedlings in the progenies of the variegated plants, the others being apparently green. When these green seedlings are transplanted into the paddy-field in order to allow their later growth, the majority grow up to be variegated and a few remain normal. The average proportion of white, variegated and green plants are 64.5 percent, 30.8 percent and 4.8 percent respectively. The albinos die soon after their germination. The green plants, which appear in the progeny of the variegated rice, breed invariably true to the type, while the variegated sisters repeat the occurrence of three forms in their offspring. With such an hereditary nature we cannot think of Mendelian segregation or ever-occurring factorial mutation. KONDÔ and his collaborators also made a hybridization experiment. They made a cross of variegated by green, from which they obtained variegated F_1 plants and bred an F_2 consisting 77.6 percent of albinos, 18.0 percent of variegateds and 4.4 percent of greens. This result is principally the same as that obtained by inbreeding the variegated rice in question. In the reciprocal crossing, they obtained only green F_1 plants. Therefore, the heredity of variegation in rice is maternal. The experiment C made by them gave quite a different result, though practically the same as in my case. My view of this is that it is due to ordinary non-Mendelian inheritance. KONDÔ and others regarded this and the former as equally ordinary non-Mendelian cases and their quantitative difference in the progeny was attributed to the occurrence of sterile flowers. The experiment C was made with variegated rice which was

[1] Their Japanese paper was published in the Journal of Scientific Agricultural Society, No. 277 pp. 443–462, 1925.

semi-sterile at the same time. The albinos obtained from four variegated plants were only 4.9 percent of their offspring, the others being pure green without exceptions. In their opinion this result is explained as follows:

"Vielleicht gibt es sehr viele Blüten, welche die Eigenschaften der Albino und zugleich die der Sterilität besitzen, was die Minderung der Prozentzahl der Albino bei Versuch C verursachen könnte" (loc. cit., p. 301).

The principal diversity of two variegated types of rice lies in the considerable difference of the frequency in appearance of variegated individuals in the succeeding generations. The genetic type of the variegation treated by KONDÔ and his collaborators in their experiments A and B does not seem to be thoroughly clear from the theory proposed by them. In my opinion, the case is complicated by the habitual occurrence of plastid mutation as shown in barley, therefore we meet with another not common case of non-Mendelian inheritance. The "unusual" type of variegation of rice has no connection with any factors contained in the chromosomes in its development as well as in its inheritance and the specimens carry quite normal genetic chlorophyll factors. The qualitative peculiarity of the cell-component of the variegateds in rice is due to the particular nature of the plastids themselves. The plastids may be normally green, containing ample chlorophyll, but it is their qualitative peculiarity that they are apt to alter themselves into white plastids. The habitual plastid mutability of this case is caused by the nature of the plastids themselves and this gives a contrast to the "special" form of the variegated barley due to the manifestation of a definite genetic fator, which stimulates the frequent occurrence of the plastid mutation. If we represent the abnormal green plastid by the symbol g', an inconstant state, it at times transforms itself into w, which has an analogy to the white plastid of the "special" form of barley in its hereditary quality. As a result of the frequent occurrence of $g' \rightarrow w$, the white stripes will appear on the foliage and ears by the sorting out of cells, which contain only white plastids, and their multiplication in the course of plant development. These cells in turn produce albino seedlings in their offspring. The remarkable divergence in the proportions of albino seedlings between rice and barley, in which the respective average counts show about 65 percent and 7 percent, will roughly indicate the difference in the frequency of plastid mutation occurring in the mother plants. The absence of all white leaves and ears is quite comparable with that observed in the variegated barley of the "special" type. The frequency of the segregation of albino seedlings is rather fixed,

though it varies to some extent in different samplings, and this is owing to the fact that all ears are finely variegated, due to the frequent occurrence of plastid mutation. If the plastid mutation occurs also in the early ontogeny of an individual or the rudiment of an ear or a leaf and the frequency of alternation is very low or entirely absent, we should have no such rather fixed proportion in the segregation of albinos. The white stripes run longitudinally on leaves and stems extending to the ears; this tells the direction of cell division in the ontogeny of Gramineae; that is longitudinal divisions generally take place early, while the transverse divisions are on the whole late. Such a mode of organ formation will limit the extension of the breadth of white stripes, as in the cells forming rudiments of leaves and ears the plastid mutation does not generally occur, but naturally it helps to regulate the average proportion of the segregation of albino seedlings.

The appearance of green plants among the progeny of the variegated rice of the "unusual" form is, in my opinion, in accordance with the plastid mutation of $g' \rightarrow g$, the latter being the non-mutable state. The low proportion of the green offspring seems to be partly connected with the small distribution of green-glumed flowers in the panicle of the variegated rice, the fact suggesting a limited period at which the plastid mutation of $g' \rightarrow g$ occurs partly due to the low frequency of its mutability, though the occurrence appears to be habitual. Among the pedigrees of KONDÔ and others, the family E/1/2/3/4 contained 22.5 percent of green individuals; this seems to be due to the qualitative alteration of a plastid or plastids in relatively early plant growth.

According to KONDÔ and his collaborators the original "unusual" variegateds, with which they carried on their experiments above cited, made their appearance as seed variation. The existence of such plants among pure green pedigrees may on the other hand be attributed to simultaneous or successive alternation of the plastids contained in an egg-cell. Supposing that the plastids of one egg-cell are all transformed from g to g', the cell could develop into a variegated plant after fertilization, since the pollen contributes no plastids to the egg in this species.

Before the publication of KONDÔ, TAKEDA and FUJIMOTO's paper, TAKEZAKI (1922) discussed the genetics of variegated rice, the transmission of which can be identified with the "unusual" form of the former investigators. Strictly speaking, TAKEZAKI's result was verified by them. TAKEZAKI interprets his case in the ordinary non-Mendelian mode of inheritance, but it is equally due to the peculiar nature of plastids contained in his variegated rice. As regards his experiments I will especially

cite the results obtained by crossings, which were made in both reciprocal ways and traced up to F_2. The F_1 plants obtained by the cross, variegated (♀)× green (♂), consisted mostly of variegateds, the rest, 20 percent, being albinos. At the seedling observation of F_2 TAKEZAKI found 42 percent albinos, the others apparently green, which later turned into variegated plants containing 7.15 percent of green sisters.

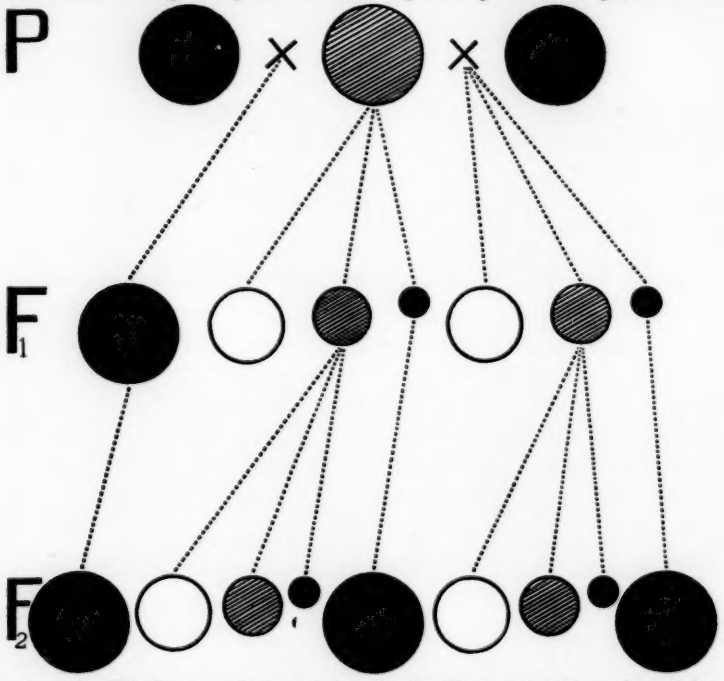

FIGURE 2.—This diagram as in figure 1 shows the hereditary mode of the variegated rice of the "unusual" type.

In the reciprocal crossing he obtained pure green F_1 and F_2. With his experiments the evidence of the maternal inheritance is quite clear. The genetic nature of the "unusual"-typed variegated rice is shown graphically in figure 2, which is drawn in conformity with the theoretical explanation based on data obtained by TAKEZAKI, KONDÔ, etc.

IV

The non-Mendelian variegation is generally due to a mixture of abnormal plastids which have changed from the normal. The sporadic

plastid mutation occurs at times in various plants. In these cases, the green branches are often produced by the sorting out of green plastids in cell multiplication. If the plastid mutation is frequently repeated the green branches cannot remain as such owing to the successive occurrence of albinotic plastids, as demonstrated by barley and rice. I have called such cases "special" or "unusual" in contradistinction to the ordinary non-Mendelian variegation. There are several variegated plants which can be considered as analogous to the not-common class. The following garden varieties of coniferous plants came under my observation, and I have met with several similar cases in the other variegated plants, a discussion of which will be left for another opportunity.

Chamaecyparis obtusa, variegated.

The variegation occurs in creamy yellow color. The development of the yellow parts on the variegated plant is abnormal, resulting in greatly contracted branchlets.

Ch. obtusa var. *breviramea*, variegated (Jap. name "Shônosuke-Hiba").

Greenish, creamy yellow variegation occurs on the foliage. Yellow branchlets of abnormal development are frequently produced. In this variety pure green branches are produced, which occurrence, however, cannot be comparable with that of yellow branches in frequency.

Ch. pisifera var. *breviramea*, variegated (Jap. name "Wakoku-Hiba").

This form has yellow patches on the foliage. The yellow parts have an arrested growth with relatively massive branchlets. Sometimes pure green branches appear on this variety.

Ch. pisifera var. *plumosa*, variegated.

White branchlets occur here and there on the plant. The variety seems to be rare in our gardens, so far as my observations go. I observed three individuals of this form, each growing about 1.5 metres high, but found no green branches. In another form, white branchlets occur in a pepper-and-salt pattern among the green foliage, with green sports appearing at intervals. The foliage of the latter variegated form, however, is somewhat contracted and has a delicate appearance, which distinguishes it from the former.

Ch. pisifera var. *filifera*, variegated.

The variegation occurs in yellow color. So far as my observation went, no green sports were found.

Juniperus chinensis, variegated.

A form with white variegation is found in the "Byakushin" type, in which needle-like foliage occurs abundantly. The other forms belonging to the "Ibuki" type, in which scale foliage occurs abundantly, are variegated with yellow or creamy white. The yellow or white branchlets are found very often in these varieties, probably in the most frequency among the variegated forms of the coniferous plants listed here. The green branches on these "Ibuki" forms are due to bud-sports, but not to the sorting out of the homogeneous green plastids, as is the case in the yellow or white branchlets. The green branches make usually very vigorous growth. In the yellow-

variegated form the yellow branchlets make somewhat strong growth in size compared with those of the white-variegated form, and the golden patches on the foliage is very evident by their frequent occurrences.

J. chinensis var. *procumbens*, variegated.

The variegation occurs in white, like that of the "Byakushin" type above listed, but the occurrence of white branches in the latter form is somewhat lower in frequency. In these two forms no green sports occur, so far as my observations go.

In these plants, the variegation is very evident, without any intergradation of color. The patch-work is generally maintained throughout the plant growth. Since the white or yellow patches occur in a coarse degree the vegetative segregation of abnormal plastids at cell multiplication must easily take place, resulting in yellow or white branchlets. If the variegation is due to the mixture of abnormal plastids and the plastid mutation is not repeated, the segregation of the green branches should be very frequent, though it never occurs by such a way in actual cases. Hence I consider them as analogous to the so-called "special" or "unusual" genetic form of variegation, in which albinotic plastids occur repeatedly by mutation.

As I did not raise the progeny of these variegated plants or hybridize them with their respective normal greens, I cannot draw a conclusion as to whether the cases are of the Hordeum or of the Oryza or of any other type. Some variegated plants shown in the list, however, sometimes bear green branches, which are considered to be produced by vegetative variation instead of segregation, as they are hardly comparable with white branches in frequency of their occurrence. The green branches make vigorous growth and become permanently green.

An interesting fact which came to my attention is the characteristic of the green reverting branches occurring on a white-variegated form of *Chamaecyparis pisifera* var. *plumosa*. The green sporting branches do not have a foliage habit quite similar to the variegated form, the foliage of which assume somewhat small and delicate appearance. This delicacy of the foliage may be due to the influence of genetic substances producing variegation. In the green sports, the genetic condition for chlorophyll production is recovered, and the branches, which live upon the delicate, variegated form, revert to the typical foliage of *Ch. pisifera* var. *plumosa*. Another variegated form of this variety, on which I failed to observe green sports, has, however, quite typical foliage. The dissimilarity in the type of foliage between these two variegated forms probably is caused by the difference of a genetic condition or genotype, by which the variegation is produced. Green sports make generally

vigorous growth compared with variegated, and they are wont to overcome the variegated stocks in growth. The undergrowth of the variegated branches is partly due to their genetic component by which the variegation is manifested, and partly to the frequent occurrence of the albinotic branchlets.

The color and growing habit of the albinotic branches on the variegated forms of the not-common type in various plants are not the same and sometimes very different, owing to the different nature of the factors or the unstable green plastids contained in the cells of the variegated forms at issue.

V

The habitual plastid mutability in the Hordeum type is due to the special quality of a definite factor and also due to a peculiarity of the plastid itself in the Oryza type. In both cases, the frequency of plastid mutability is affected by the influence of ontogenical stages of the plants. In barley the green hybrids carrying the *GG'* constitution give progenies containing green and variegated individuals at a 3:1 ratio, with no albinos. The dominance of the factor *G*, therefore, is complete. In addition to this, the plastid mutation does not seem to occur in gametogenesis or in the course of the primitive somatic development, and we have no chance of obtaining albino seedlings in the progeny of such heterozygous greens. The factor *G'* causes the frequent production of white plastids through the transformation of $g \rightarrow w$, and the factor itself is highly constant, so we have no green individuals among the progeny of the variegated barley in question. In rice, on the contrary, the green individuals appear almost habitually in the progeny of the variegated form of the "unusual" type. This is due to the change of the plastid itself from *g'* to *g*, but no factor concerns itself with this event. The frequent alternation of plastids occurs in two directions, $g' \rightarrow w$ and $g' \rightarrow g$, but these two differ in the frequency of occurrence and in the period at which the plastid mutation takes place. The difference in the latter suggests a delicate response of the plastids to the ontogenical environment of the plants in their transformation. The old stems of the variegated variety of *Juniperus chinensis* sometimes give buds which, however, are purely or almost white in the majority of cases, so far as my observation was made. The shooting branches of the variegated *Serissa foetida*, the quality of which is considered to be not common in its variegation, generally bear leaves with small white patches nearly green, while the lateral branches frequently are purely or almost white, especially with the plant

cultivated in a pot. The facts above cited show the delicate response of the plastids as to their tranforming frequency in plant growth. KONDÔ, TAKEDA and FUJIMOTO's observation on mowed stocks of the variegated rice of the "unusual" type gives another confirmation of this relation. Their experiments revealed that "aus diesen Wurzelstocken trieben neue Pflanzen, und die Verfasser haben beobachlet, dasz aus den Wurzelstocken der gestreiften Pflanzen jedesmal in noch höherem Grade weissgestreifte . . . . hervorkamen" (loc. cit. p. 312). The frequent occurrence of white or heavily variegated branches on the variegated plants of the "unusual" type may prove that simultaneous or successive plastid mutation takes place in the rudimentary cells of buds. This happening sustains my postulate as to the origin of the "unusual" type of rice with variegation, as tentatively suggested on a previous page.

Generally speaking, the segregation of albinos among a progeny propagated by the self-fertilization of heterozygous or variegated plants can be summed up as to their genetic types as follows:

I. Mendelian case (figure 3-A).
II. Non-Mendelian case.
  1. Ordinary form (figure 3-B).
  2. Not-ordinary form.
    a. Hordeum type (figure 3-C)
    b. Oryza type (figure 3-D)

In Mendelian segregation the albinism is due to an albino factor, which affecting the plastids make them outwardly colorless. The essential quality of albinotic plastids, however, is perfectly normal. Figure 3-A shows diagrammatically such a relation. The albinism of the ordinary form of non-Mendelian inheritance is due to the massive segregation of white plastids which character is manifested by their own abnormal quality and has no concern with any chlorophyll factors in their manifestation. Figure 3-B represents this relation and the origin of white and green segregates. The albinism of the "special"-typed barley is the same expression as that of the ordinary non-Mendelian case, due to the manifestation of the *w* plastids. But the cause of the successive occurrence of the white plastids is due to the factor *G'*, which at times alters the essential quality of plastids from *g* to *w*. Figure 3-C is drawn to demonstrate this relation. In the Oryza type the albinism is also due to the manifestation of the *w* plastids, the cause of the alteration, however, being attributable to the *g'* plastids themselves which may be phenotypically green, but lacking full constancy. The alteration of the plastids, therefore, occurs quite out of control of the genetic factors but

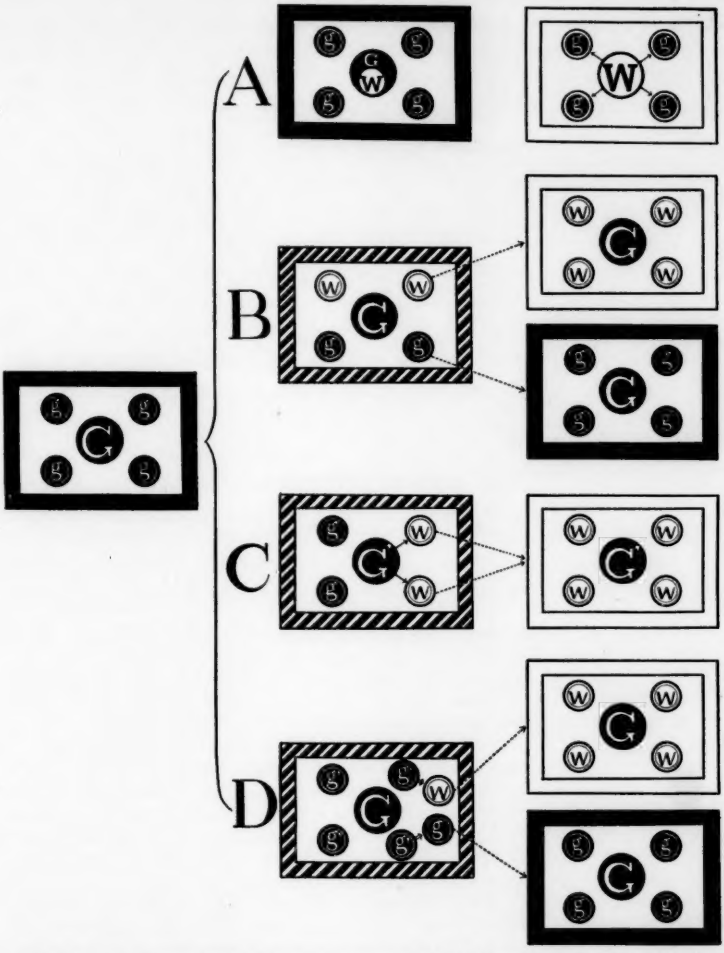

FIGURE 3.—A *schema* showing the genetic types of plastid inheritance, in which albino seedlings are segregated. A and B, Mendelian and ordinary non-Mendelian cases respectively; C, Hordeum type of the "special" form; and D, Oryza type of the "unusual" form. The left square shows the normal green condition. The squares in the center of the figure represent the variegated condition (heterozygote in A which is the Mendelian case). The squares to the right show the albinotic and the green plants sorted out from the respective central squares. Each square shows the model of a cell which contains one nucleus (the large central circle) and four plastids (small peripheral circles). The frame of the cell represents its color, the solid being that of a green cell; the barred that of a variegated cell; and the blank that of a white cell. The genotype of the cell is shown by the color and symbol given in its nucleus. The color of the rim of the plastid indicates its character, while the color and symbol of the center show its real quality.

directly from g' to w. The green individuals among the progeny of the variegated rice in question owe their origin to a similar process; namely, they originated by $g' \rightarrow g$. See figure 3-D, which shows these relations diagrammatically.

VI

The albinotic plastids with albino property usually have a fair constancy as to their quality, and the white cells propagate into purely white tissues or organs by their successive divisions. The common forms of the chlorophyll periclinals and reversals have purely white or yellow skins or cores, which character is non-Mendelain in inheritance, due to the constancy of the quality of the plastids contained in the cells. In some less-known chimaera plants, such as the periclinal variety of *Gardenia florida*, the white skin, which covers the green core, has green ticks and patches, the white tissues being never pure in color. CHITTENDEN (1925) published some interesting results gained in his experimental series of the chimaerical plants, Hydrangea and Pelargonium The variegated forms, on which he made genetical and anatomical studies, contain some complicated chimaeras analogous to the variegated Gardenia above cited. In Hydrangea, he observed three variegated forms,[2] which, according to his denomination, are Types A,[3] B and C, the latter

[2] The common white-over-green form is found in our Hydrangeas, but it was not present in CHITTENDEN'S collection.

[3] To cite CHITTENDEN'S statement, this type "has the stems and the centres of the foliar organs solid white; the latter have a broad margin of solid green. This form, though frequently producing sports wholly green, shows otherwise great regularity of distribution in the type foliage" (loc. cit., p. 44). Such a sort of variegation or "peripheral" chimaera witnessed in some garden plants, is, according to my opinion, probably nothing but a periclinal, in which the yellow or white core seems to contain a toxin that affects the healthy plastids of some outer green tissue by assuming temporarily yellow or white color. The leaf margin of the variegated form, however, does not receive the influence of the toxin secreted by the core, and for that reason the leaf is marked with a green-and-yellow or green-and-white peripheral pattern. This view will explain quite satisfactorily the production of bud-sports, the behavior of which run remarkable parallel with the common periclinals. Among CHITTENDEN'S zonal Pelargoniums, Freak of Nature and Happy Thought seem to be modified periclinals of the green-over-white or green-over-yellow type, in which the white or yellow core affect the green skin at their outer surfaces on both sides of the leaves, resulting in peripheral chimaeras. Chittenden, however, seems not to agree on this view, as he states that "Happy Thought is evidently not a periclinal, for the central *aurea* tissue of the leaves is not covered by any other" (loc. cit., p. 51). This question will be solved by the assumption of a toxic substance, which affects the green plastids in dissolving their chlorophyll, secreted by the albinotic cells or plastids. If these chimaerical forms are Type A of Hydrangea and Freak of Nature and Happy Thought of Pelargonium, with which CHITTENDEN made breeding experiments, they should produce results like those expected from the breeding of the green-over-white or green-over-yellow periclinals. Actually CHITTENDEN'S description of the breeding aspect of the A-typed Hydrangea runs: "This green marginate Type A×green (Type D) throws only

two of which will be treated in this text. Type B[4] is a periclinal with green core which is covered by white skin containing green patches. According to him the breeding aspect of this form is as follows: "When a B Type is fertilized by a green Type D male, it gives both white and green seedlings; when, however, it is used as a male on a green Type D female, only green seedlings results" (loc. cit. p. 44). CHITTENDEN's Type C, however, is a periclinal with green core covered by white skin containing yellow patches instead of green ones.[5] His breeding experiment gave the result that "When fertilized with pollen of a green (Type D), non-viable seedlings, some yellow and some white, result, whereas in the reciprocal cross, only green seedlings are produced" (p. 45). These and other results with Hydrangea led him to conclude that " . . . . the seedlings, regardless of chlorophyll distribution in the male parent, reproduce only the types composing the actual leaf margin of the female." If so how can we understand the origin of green or yellow patches in white tissues? In my opinion, they are probably due to mutations of the albinotic plastids to the green ones, which distribute into massive cells after cell multiplication. The quality of the albinotic plastids contained in the white tissues of these periclinals is not manifested by nuclear substances, but due to the plastids themselves. The mutating green plastids may be induced by the alteration in the quality of the plastids. According to CHITTENDEN, Freak of Nature, whose stems, leaf-stalks and centres of leaves are white, produces white branches having minute green ticks at times on the margins of the stipules and rarely on the

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green seedlings" (p. 44). By selfing the flowers of Freak of Nature he obtained eight seedlings, which were purely green. These results quite satisfy my view. Happy Thought gave segregating progeny in his examination, but, in my opinion, this was induced by the heterozygous genotype of the green skin of the variety. ROTH's experiments (1927) happened to prove the impure genotype of Happy Thought. So the conspicuous segregation does not contradict the periclinal view above proposed. The fact that the adventitious buds formed on the roots of Freak of Nature were always white in color will give an additional confirmation for the hypothesis, according to which the variety consists of periclinal mosaic tissues.

[4] CHITTENDEN states: "This is a white-over-green periclinal, but differs from ordinary periclinal types in having lobes of solid green, without a white skin, irregularly distributed along the margin of the leaves. These lobes occur as outgrowths from the leaf margin. They project from the general contour of the leaf, and their growth is more ample than that of corresponding parts covered by the white skin. Sometimes such lobes are few, but generally several are borne by each leaf. The marginal lobes are usually separated by a white interval from the general body of the green core, and, though seeming occasionally to touch the inner green, they are separate developments, distinct from the core" (loc. cit., p. 44).

[5] According to him, "This form is similar in chlorophyll distribution to Type B, but the lobes consist of pale yellow instead of green tissue. Here also the lobes are areas of rapid growth, and all degrees of lobing occur" (loc. cit., p. 44).

limbs of the leaves. His statement is—that "From such a branch, two wholly green seedlings were once raised; presumably they traced their origin to such a—potentially—green area" (p. 49). I regard the green ticks on the white branches to be due to their mutating origin. Golden Brilliantissima, one of periclinal Pelargoniums, whose behavior was examined by CHITTENDEN, is a form similar to Type B of his Hydrangea. From this form he obtained seven seedlings, all yellow in color, though white ones also may be expected to appear. The absence of white seedlings may possibly not be an incidental result, as he states: "Golden Brilliantissima sets seed badly; a large number of pollinations only produced 156 doubtful-looking seeds, of which 7 germinated" (p. 52). According to the experiment made by CLAUSEN (1927), the seeds of Viola. containing white embryos, do not germinate. At present, however, we cannot draw any conclusion beyond a suggestive statement, and the solution of the problem can confirm a result of further experiments.

CONCLUSION

The inheritance of variegation of the ordinary non-Mendelian type in both barley and rice is closely connected with their particularly coarse pattern of variegation. It is suggested that variegation in these cases is due to sporadic mutation of the plastids themselves, which change from green to albinotic. When the abnormal plastids are later propagated and distributed in the tissues through cell multiplication variegated or albinotic bud-sports are produced. The factorial mutations are generally put into two categories of sporadic and habitual occurrences (IMAI 1927), and likewise the plastid mutations are not confined to sporadic occurrences.

SÔ (1921) studied thoroughly the genetics of a finely variegated variety of barley and cleared up its special behavior. The variegated character is transmitted as a simple recessive to the green. The white stripes on the variegated foliage, however, are due to the distribution of white plastids, which have changed their own quality permanently by plastid mutation in the presence of certain recessive genetic factors. The plastid mutation in this case occurs quite habitually and it results in a fine pattern of variegation in this variety. The characteristic of the recessive factor which is contained in the cells of this variety, therefore, is that it alters at times the essential quality of some of the plastids from normal green to colorless. In this case, the plastid mutation occurs so frequently in the course of the development of the plant body that not a single individual or even a leaf or an ear, which is free from the variability or

self-green, is produced. This is interpreted to mean that the variegated character is transmitted itself as a simple Mendelian recessive to the green. The transmission of the white plastids, however, is maternal and does not follow Mendelian rules. So far as I can judge, the mutability of the plastid is not distributed over the whole cycle of the plant development, but the mutable period begins at a later embryonic stage and ceases in later somatogenesis. This assumption explains the particular points of this variegation. The genetics of this variegated barley, therefore, cannot be simply comparable with the ordinary variegation, the case being "special" in its behavior.

The variegated races of rice studied by KONDÔ, TAKEDA and FUJIMOTO (1927) are grouped under two classes in regard to their hereditary type: In one the variegateds reappear in a large proportion of the progeny, in the other practically no variegateds are present but segregation produces green and white seedlings. The latter is identified with the ordinary non-Mendelian case. In the former case, they obtained white, variegated and green progenies in the proportion of 64.5 percent, 30.8 percent and 4.8 percent respectively. The green plants breed invariably true to the type, whereas their variegated sisters repeat the previous performance. The variegation of rice is transmitted maternally in hybridization with green, and the variegated F_1 gives white, variegated and green F_2 segregates in roughly the same proportion as when the variegated plants are self-propagated. They, however, regarded all their cases equally as ordinary non-Mendelian inheritance. In my opinion, the present case is of a not-common form of non-Mendelian inheritance, that is, the plastid mutation occurs quite habitually like the variegated barley of the "special" type. The habitual plastid mutation is not due to any genetic factors contained in nucleus, but to the special quality of plastids themselves contained in the cells of this variegated rice. The plastids alter their quality from green to white by their own instability. The plastid mutation in this case, as in the variegated barley of the "special" type, seems to begin in the late embryonic stage and to cease in the late somatogenesis, including gametogenesis. The appearance of green plants among the progeny of the variegated rice of the "unusual" form is, in my opinion, in accordance with the plastid mutation of the unstable plastids to their non-mutable state. Before the appearance of KONDÔ and his collaborators' paper, TAKEZAKI (1922) published the similar data with variegated rice.

So far as I can judge, there are not a few variegated plants which may be classified under the not-common form in their genetic behavior, among which I have listed several coniferous plants. The plastid mutation

occurs habitually from green to colorless in these plants. Sometimes green branches, due to mutation, are produced on some of these variegated varieties.

The less-known periclinal chimaera plants which have green cores covered by white skins containing green patches, which CHITTENDEN (1925) studied, are interesting material for an investigation of plastid mutation. According to my view, the origination of green or yellow ticks in the white tissues of his chimaera plants may be due to the plastid mutation, occurring quite habitually, from white to green or to yellow.

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